Ecology of the Genus *Burkholderia* in the Soil

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Nejc Stopnisek

aus

Slowenien

Promotionskomitee

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Zürich, 2014

Ago

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Summary

The genus *Burkholderia* consists of 86 phylogenetically closely related species that are metabolically highly versatile. This enables them to be ubiquitously present in the environment as free-living bacteria as well as in antagonistic, mutualistic or symbiotic associations with plants, fungi and animals. Soil is the environment that contains the largest pool of *Burkholderia* diversity. Soil *Burkholderia* are involved in a large number of processes, such as decomposition of organic matter, detoxification/removal of pollutants or nitrogen fixation. Even though these processes are well understood, there is still a lack of knowledge regarding the ecology of *Burkholderia* in the soil.

To better understand the environmental parameters affecting *Burkholderia* distribution and abundance in the soil we investigated the relative abundance of the genus *Burkholderia* in the soil at a trans-continental and at a local scale and correlate the obtained abundances with environmental parameters. The results showed that *Burkholderia* relative abundance was significantly influenced by soil pH. In contrast to most bacteria, *Burkholderia* favored low pH environments and were undetectable in neutral and alkaline soils. However, while their relative abundance was influenced by the soil pH, their diversity was not. Moreover, *in vitro* growth experiments revealed that although they are undetectable in neutral or high pH soils, *Burkholderia* are able to grow at pH values as high as 9, suggesting that beside abiotic factors such as pH, biological interactions such as the presence of interacting partners might contribute to *Burkholderia* abundance and diversity.

In a second part of the thesis, we investigated the interactions of *Burkholderia* with fungi. Interactions between fungi and selected *Burkholderia* strains are described, but less is known about their relevance in explaining *Burkholderia*'s biogeography and preference for low pH soils, or the mechanisms underlying such interactions. We used a proteomic approach to gain first insight into the physiological changes occurring in *Burkholderia glathei*, a common soil bacterium, while interacting with two fungi, *Alternaria alternata* or *Fusarium solani*. Interestingly, the proteome of *B. glathei* underwent similar changes with both fungi, and these changes revealed a highly beneficial effect for the bacterium, which apparently derived much of its carbon,

nitrogen and phosphate from the fungi. Additionally, co-occurrence network analysis and growth experiments revealed that these associations between *Burkholderia* and fungi are very common in soils and occur with a broad range of fungal partners.

In the third part of the thesis, we investigated the interaction of *Burkholderia* with plants, with the focus on the role of oxalate degradation in root colonization. Oxalotrophy (the use of oxalate as a carbon source) was a feature restricted to plant-beneficial *Burkholderia* strains and absent from the pathogenic strains. To evaluate whether oxalotrophy was involved in rhizosphere competence, we compared the colonization of lupin and maize by a mutant strain of *B. phytofirmans*, which was impaired in oxalate degradation, with that of its wild type. The results showed that the wild type strain had a significant advantage in root colonization compared to the mutant strain, suggesting that oxalate degradation plays an important role in biological interactions of *Burkholderia* with plants.

All three studies have extended our knowledge of the different lifestyles of *Burkholderia* in soils and have unveiled some part of their immense ecological potential.

Zusammenfassung

Die Gattung *Burkholderia* besteht aus 86 phylogenetisch eng miteinander verwandten Spezies, welche metabolisch sehr vielseitig sind. Diese Vielseitigkeit ermöglicht es ihnen in der Umwelt als frei lebende Bakterien sowie als Antagonisten, Mutualisten oder Symbionten von Pflanzen, Pilzen und Tieren omnipräsent zu sein. Das grösste Reservoir an *Burkholderia* Diversität ist im Erdboden zu finden. Im Erdboden sind *Burkholderien* an einer grossen Anzahl von Prozessen, wie an der Zersetzung von organischem Material, Entgiftung und Abbau von Schadstoffen sowie an der Fixierung von Stickstoff beteiligt. Obwohl diese Prozesse gut erforscht und verstanden sind, gibt es noch beträchtliche Wissenslücken bezüglich der Ökologie von *Burkholderien* im Erdboden.

Um die Umweltparameter, welche die Verteilung und Häufigkeit von *Burkholderien* im Erdboden beeinflussen besser verstehen zu können, untersuchten wir die relative Häufigkeit der Gattung *Burkholderia* im Erdboden im transkontinentalen und lokalen Massstab. Die ermittelte Häufigkeit wurde anschliessend mit verschiedenen Umweltparametern korreliert. Die Resultate zeigten, dass die relative Häufigkeit von *Burkholderia* vom pH Wert des Erdbodens signifikant beeinflusst wird. Im Gegensatz zu den meisten andere Bakterien, bevorzugten *Burkholderien* Umgebungen mit niedrigem pH Wert und waren in Erdböden mit neutralem oder alkalischem pH Wert nicht zu detektieren. Obwohl die relative Häufigkeit vom pH Wert des Erdbodens beeinflusst wurde, traf dies nicht auf die Diversität der *Burkholderien* zu. *Burkholderien* waren in Erdböden mit neutralem und alkalischem pH Wert zwar nicht deketierbar, aber *in vitro* Wachstumsexperimente zeigten, dass sie trotzdem bis zu einem pH Wert von 9 wachsen können. Dies deuted darauf hin, dass neben abiotischen Faktoren wie pH, biologische Interaktionen, wie etwa das Vorhandensein eines Interaktionspartners zur Häufigkeit und Diversität von *Burkholderien* beitragen könnten.

Im zweiten Teil der Dissertation untersuchten wir die Interaktion von *Burkholderia* mit Pilzen. Interaktionen zwischen Pilzen und ausgewählten *Burkholderia* Stämmen sind in derLiteratur beschrieben. Jedoch ist nur wenig über die Relevanz solcher

Interaktionen für die Biogeographie von *Burkholderia* sowie deren Präferenz von Erdböden mit niedrigem pH Wert bekannt. Entsprechend gibt es auch keine Untersuchungen der Mechanismen welche diese Interaktionen ermöglichen. Um erste Einsichten in die physiologischen Veränderungen von *Burkholderia glathei* bei Interaktion mit zwei verschiedenen Pilzen, *Alternaria alternata* und *Fusarium solani* zu bekommen, wurde von uns ein Proteomik-basierter Ansatz gewählt. Das Proteom von *B. glathei* zeigte ähnliche Veränderungen bei der Interaktion mit den beiden verschiedenen Pilzen, welche nützliche Effekte auf das Bakterium hatten. So scheint *B. glathei* einen großer Teil seines benötigten Kohlenstoffs, Stickstoffs und Phosphats von den beiden Pilzen zu beziehen. Zusätzlich haben Kookkurenz Netzwerk Analysen und Wachstumsexperimente gezeigt, dass die Assoziationen von *Burkholderien* und Pilzen im Erdboden weit verbreitet sind und mit einer Vielzahl unterschiedlicher Pilze entstehen können.

Im dritten Teil der Dissertation untersuchten wir die Interaktion von *Burkholderien* mit Pflanzen. Dabei wurde der Fokus auf den Oxalatabbau während der Wurzelkolonisierung gelegt. Oxalotrophie (Verwendung von Oxalat als Kohlenstoffquelle) war eine Eigenschaft von *Burkholderia* Stämmen mit positiven Auswirkungen auf Pflanzenwachstum, während pathogene Stämme keine Oxalotrophie aufwiesen. Um beurteilen zu können ob Oxalotrophie an der Rhizospherenkompetenz beteiligt ist, verglichen wir die Kolonisierung von Lupinen und Mais zwischen einem *Burkholderia phytofirmans* Wildtypstamm und einer Mutante, welche nicht zum Oxalatabbau fähig ist. Es zeigte sich, dass der Wildtypstamm einen signifikanten Kolonisierungsvorteil gegenüber der Mutante aufwies. Dies legt nahe, dass der Abbau von Oxalat eine wichtige Rolle in der Interaktion von *Burkholderia* mit Pflanzen spielt.

Alle drei Studien haben dazu beigetragen unser Wissen über die verschiedenen Lebensweisen von *Burkholderien* im Erdboden zu erweitern und konnten einen Teil des immensen ökologischen Potenzials dieser Bakterien aufzeigen.

Abbreviations

bp Basepair

BCC *Burkholderia* cepacia complex

BSA bovine serum albumine

° C degree Celsius

Cd Cadmium

CFU Colony forming units

DMSO Dimethylsulfoxid

DNA Desoxyribonucleic acid

dNTP Desoxynucleotidetriphosphates

E. coli Escherichia coli

EDTA Ethylendiamin-Tetraacetat

e.g. for example (Latin: exempli gratia)

EPS Extracellular polymeric substances

et al. and others (Latin: et alii)

EtBr Ethidiumbromid

EtOH Ethanol

g Gram

GC Guanine, Cytosine

GFP green fluorescent protein

h Hour

i.e. that is (latin: id est)

Km Kanamycin

l Litre

LB Luria Bertani medium

m Meter

M Molar

MEA Malt extract agar

min Minutes

mM Milimol

MQ milli Q water

n nano (10^{-9})

NRPS nonribosomal peptide synthesis

OD optical density

OTU Operational taxonomic unit

PAH Polyaromatic hydrocarbon

PCR Polymerase Chain Reaction

PCB Polychlorobiphenyl

PIA *Pseudomonas* isolation agar

rRNA ribosomal ribonucleic acid

s Second

SDS Natriumdodecylsulfat

TAE Tris, Actetat, EDTA

T3SS Type 3 secretion system

% Percent

% (v/v) Volume per volume

% (w/v) Weight per volume

µ Mikro (10^{-6})

General introduction

Species of the genus *Burkholderia* are diverse, ubiquitously distributed and can be isolated from a wide range of environments. However, the main habitat of *Burkholderia* species is the soil, where they are involved in different environmental processes and in interactions with fungi, plants and animals.

The genus *Burkholderia*

Bacteria from the genus *Burkholderia* were first described in 1942 by Walter Burkholder as phytopathogenic organisms affecting carnation and onions (Burkholder 1942). In 1950, he additionally described a species, named *Pseudomonas cepacia*, which was causing rot in onions (Burkholder 1950) and became the type strain of the current genus. From the first isolations and until their own genus was proposed, most of the newly isolated bacteria were considered as members of non-fluorescent pseudomonads. In the early nineteen's, results obtained by rRNA-DNA and DNA-DNA hybridization techniques and fatty acid analysis provided sufficient support for proposing *Burkholderia* as a new genus of seven species from the *Pseudomonas* homology group II (Yabuuchi *et al*. 1992).

Today the genus *Burkholderia* contains 86 distinct but highly related species (www.bacterio.net, June 2014), whose strains were isolated from various ecosystems. In the 1980s, strains of *B. cepacia* had been repeatedly isolated from cystic fibrosis patients (Isles *et al*. 1984). Taxonomic studies revealed that these opportunistic pathogens belonged to five closely related species and were referred to as the *Burkholderia cepacia* complex (BCC). The BCC currently consists of 17 species that share 98-100% similarity in their 16S rRNA gene and 94-95% in their *recA* gene sequences (Vanlaere *et al*. 2008, Vanlaere *et al*. 2009). The adaptability and metabolic versatility of species belonging to the BCC enables them not only to colonize and infect the human body but also to cause diseases in plants and animals. In contrast, species of the BCC also have plant growth promoting effects and have the ability to produce multiple antifungal compounds (Lin *et al*. 2012). Beside the opportunistic pathogens of the BCC, the genus *Burkholderia* also contains few

obligate pathogens. Among those are members of the species *B. mallei* and *B. pseudomallei*, causing glanders in animals and melioidosis in humans, respectively (Gilad 2007). Due to their pathogenic properties, *Burkholderia* have received a lot of scientific attention. The findings that *B. brasilensis* and *B. kururiensis* are able to fix nitrogen and nodulate legumes, gave this genus a strong ecological meaning, in addition to its clinical relevance (Baldani *et al*. 1997, Estrada-De Los Santos *et al*. 2001, Moulin *et al*. 2001). Later many strains of *Burkholderia* have been isolated, described and assigned to a group of environmental and plant beneficial species, to which also *B. brasilensis* and *B. kururiensis* belong. Multilocus sequence analysis of several housekeeping genes (Figure 1) clearly separates this non-pathogenic group (Group A in Figure 1) from the pathogenic *Burkholderia* clade (Group B in Figure 1) (Estrada-de los Santos *et al*. 2013). As the name already suggests, members of this group have been repeatedly found in the environment, especially in the soil where they perform a number of important processes and are found in exo- and endo-symbiotic associations with plants, fungi and invertebrates (Levy *et al*. 2003, Kikuchi *et al*. 2005, Carlier and Eberl 2012). Interestingly, strains from the same species belonging to this environmental group of *Burkholderia* have been recovered from distant geographical locations and from very different niches. *B. caribensis* strains were first isolated from a vertisol on Caribbean islands and were later also found in nodules of *Mimosa* sp. in Southeast Asia (Achouak *et al*. 1999, Liu *et al*. 2011). *B. tropica* strains have been isolated from the rhizosphere of sugarcane, maize and teosinte in Mexico, Brazil and South Africa (Reis *et al*. 2004, Perin *et al*. 2006). Similarly, *B. graminis*, *B. unamae* and *B. silvatlantica* are common rhizobacteria of maize, pasture, sugarcane, coffee and wheat in Australia, Brazil, USA and France (Estrada-De Los Santos *et al*. 2001, Caballero-Mellado *et al*. 2004, Perin *et al*. 2006, Castro-González *et al*. 2011). Beside living in the rhizosphere, *B. phytofirmans*, *B. kururiensis* M130 and *B. acidipaudis* can also exhibit endophytic lifestyles within different plants (Sessitsch *et al*. 2005, Mattos *et al*. 2008, Aizawa *et al*. 2010). Symbiosis between Candidatus *B. kirki* and *Psychotria kirki* was recently described as the first example of obligate symbiosis between bacteria and plants (Carlier and Eberl 2012).

Although very often identified in association with plants, multiple strains have been also found free-living in bulk soil. *B. caledonica*, *B. sordidicola, B. cordobensis* and *B. eburnea* have been isolated from bulk forest, agricultural and peat soils (Coenye *et al*. 2001, Draghi *et al*. 2014, Kang *et al*. 2014). *B. xenovorans*, *B. jiangsuensis* and *B. sartisoli* have been isolated from soil contaminated with either polychlorobiphenyl (PCB), methyl parathion (MP) or polyaromatic hydrocarbons (PAH) (Martínez *et al*. 2007, Schamfuss *et al*. 2013, Liu *et al*. 2014a).

With their wide geographical distribution, versatile ecological properties, biological interactions and high cultivability, *Burkholderia* have gained much attention in recent years within the scientific community. Bontemps *et al*. (2010) suggested that the genus *Burkholderia* has the potential to be used as a model to understand how pathogenesis and symbiosis have emerged, spread, evolved and segregated within a single bacterial genus.

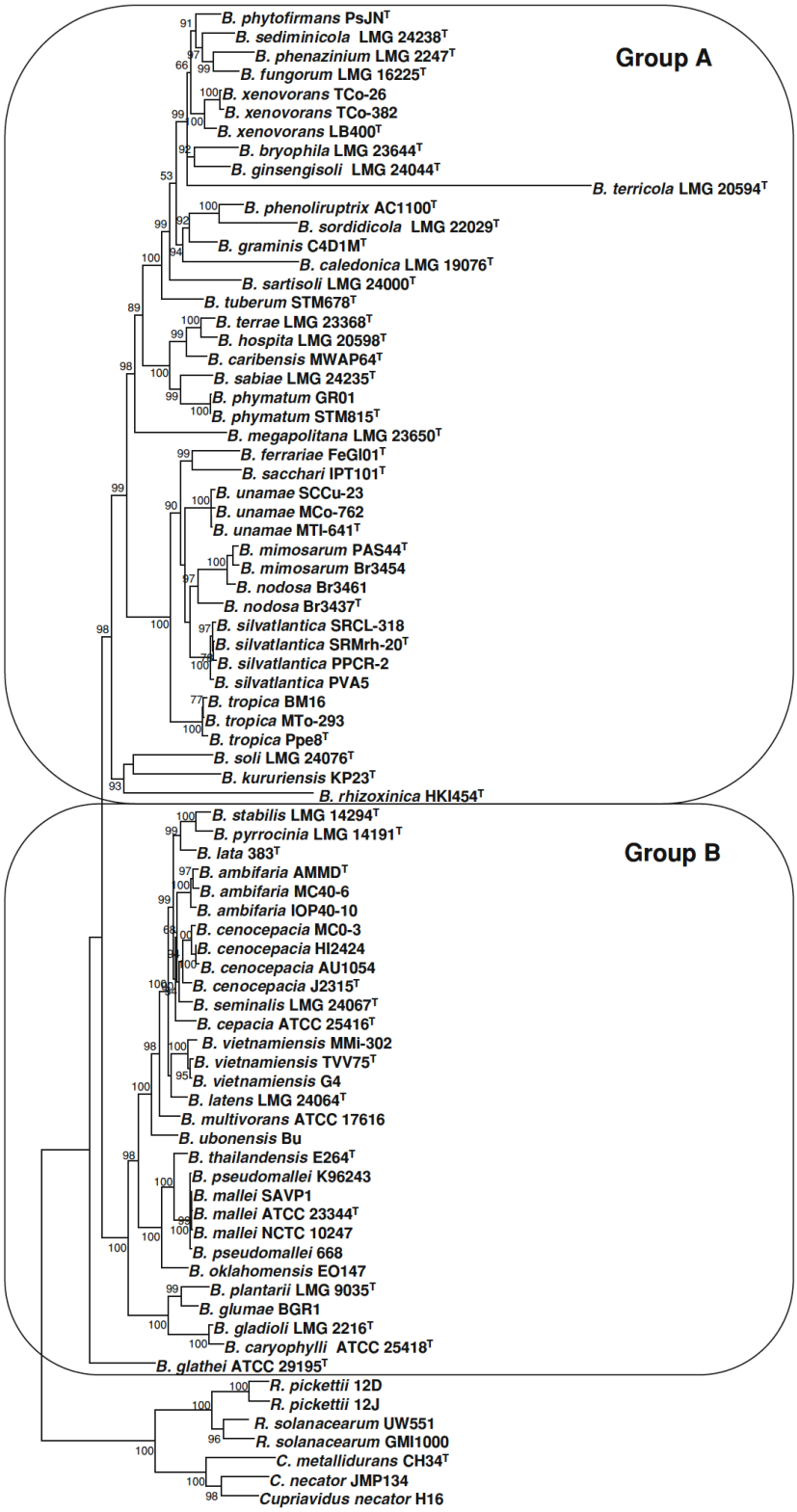

Figure 1. *Burkholderia* sp. phylogenetic tree. Maximum likelihood species tree inferred from the concatenated alignment of *atpD–gltB–lepA–recA*–16S rRNA genes, showing the phylogenetic relationship of 77 type and reference *Burkholderia* strains (from Estrada-de los Santos *et al*. (2013)).

Soil as habitat for the genus *Burkholderia*

Soils possess vast and diverse microbial communities. Bacterial abundance in the soil can range from 10^9 cells cm^{-3} of forest soil to 10^{11} in pasture and arable soils (Torsvik *et al*. 2002). With the development of novel high-throughput sequencing methods, the perception of the soil microbial diversity has changed drastically. Using DNA-DNA hybridization kinetics, Torsvik *et al*. (1990) proposed that bacterial soil communities might contain approximately 4000 species, however, using DNA pyrosequencing datasets, Quince *et al*. (2008) came to the conclusion that there might be between 20 000 and 140 000 different species depending on the soil.

The balance between bacterial populations varies according to a number of factors. From the aspect of trophic interactions two diversity control mechanisms exist: "bottom-up" and "top-down". The first includes the competition for the same nutrients and the availability of the resources, whereas the second includes biological control such as predation and grazing and takes into account the important role of viruses in these control processes (Fuhrman and Schwalbach 2003). Natural selection is another important factor; the large population sizes of soil microbes lead to the accumulation of many mutations, whereas lateral and horizontal gene transfers, as well as recombination, contribute to diversification, speciation and extinction of soil microbes (Cohan 2005). Furthermore, microbes in the soil encounter spatial and temporal heterogeneity that also contribute to changes in bacterial diversity. Temporal disturbances such as starvation, desiccation, freezing/thawing and anthropogenic activity alter environmental conditions and resource availability, which creates opportunities for the establishment of new species (Evans and Wallenstein 2014). The structural complexity of soil is important for population-level diversification because it allows resources to be partitioned and creates new niches, thereby enhancing specialization and division into distinct ecological species (Nunan *et al*. 2003). The soil matrix provides the opportunity for spatial isolation and thereby for the control of microbial diversity. Beside the soil matrix, substrate concentrations, redox potential and pH also contribute to the formation of multiple microhabitats (Or *et al*. 2007).

pH in particular has been shown to have a significant effect on soil microbial distribution, abundance and diversity. Fierer and Jackson (2006) investigated the

environmental factors contributing to microbial diversity and biogeography. They analyzed 98 soil samples collected across South and North America with a DNA-fingerprinting method targeting the 16S rRNA gene. They found that, despite the large ecosystem diversity that those samples covered, microbial diversity and richness were largely explained by soil pH. As a result, bacterial diversity was high in neutral soils and lower in acidic soils. Later, 88 soil samples from the same collection were pyrosequenced and the results supported the previous findings that pH has a significant effect on bacterial diversity (with a peak at near neutral pH) (Lauber *et al*. 2009). Interestingly, community composition, which correlated significantly with pH, was largely driven by changes in the abundance of *Acidobacteria*, *Actinobacteria* and *Bacteroidetes* (Lauber *et al*. 2009). Additionally, by influencing soil microbial communities, pH also has a potential impact on the processes that are mediated by these communities (Nannipieri *et al*. 2003, Kemmitt *et al*. 2006, Rousk *et al*. 2011). Soil pH is often correlated with important environmental factors that influence the microbial community, such as nutrient availability (Härdtle *et al*. 2004), heavy metal solubility and toxicity (Chuan *et al*. 1996), as well as plant community structure (Schuster and Diekmann 2003). Thus it is difficult to determine if the community structure is controlled specifically by pH. One method to investigate this question is to measure the pH-optimum for growth (pH_{opt}) of a bacterial community by following the incorporation of labeled substrates, such as leucine or thymidine in different pH conditions (Bååth 1998). Applying this method to a variety of soils with a wide range of pHs, Fernandez-Calvino *et al*. (2011) showed that the soil pH correlated significantly with the pH_{opt}. The pH range, in which bacterial community growth decreased by 50% was ± 2.1 units. However, the authors argued that even a 10% decrease in growth of a given bacterial species most likely would result in this species being outcompeted by better adapted ones, thus leading to the assumption that the actual pH range would be quite narrow, approx. ± 1, with a slightly wider range in acidic soils (Fernández-Calviño and Bååth 2010). One of the reasons why the pH_{opt} range is expected to be wider in acidic soils, is the occurrence of microscale niches, where the pH would be locally higher than that measured in the bulk soil.

Despite these observations, very little is known about the microbes living in particular pH ranges, especially in the acidic conditions. In addition to some bacterial and

archaeal species known to contain acidophilic strains (Johnson 1998), the only genus described to show a preference for acidic soils is the genus *Acidobacteria*, and even here, only a subdivision is acidophilic (Jones *et al*. 2009).

Burkholderia in the soil

Even though comprehensive studies investigating the whole genus *Burkholderia* have not been published yet, the reports targeting specific *Burkholderia* strains or focusing on the microbial community compositions in specific soils, suggest that *Burkholderia* might be another bacterial genus that is frequently found in low pH environments. As an important pathogenic bacterium, *B. pseudomallei* has been very intensively studied since its discovery. Early studies investigating the distribution of this species and using cultivation-based techniques revealed that *B. pseudomallei* was favored by the relatively acidic environment of rice paddy (pH 4.4 – 7.7), where strains belonging to this species have been repeatedly isolated from (Kanai and Kondo 1994). This preference for acidic soils was confirmed also in a large scale study where specific quantitative PCR, targeting *B. pseudomallei* type 3 secretion system (T3SS), was applied on DNA isolated from 809 Australian soils (Kaestli *et al*. 2009). Non pathogenic strains of the *Burkholderia* genus were also isolated from soils with a pH as low as 2.9 (Curtis *et al*. 2002). The obtained isolates were able to grow exponentially in media with a pH lower than 4, suggesting growth and not only survival in such acidic environments (Curtis *et al*. 2002). *Burkholderia* species are common members of rhizosphere communities and as such, they also have been repeatedly detected in *Sphagnum* peat bog soils that have naturally a very low pH. Representative species of such peat bog inhabitants were *B. glathei*, *B. phenazinium*, *B. fungorum*, *B. caryophylli*, *B. bryophila* and *B. megapolitana* (Belova *et al*. 2006, Opelt *et al*. 2007, Vandamme *et al*. 2007). Additionally, similar species have been found in association with the highly acidic rhizosphere of white lupin and were significantly enriched with increasing cluster root age, which is linked with drastic changes in pH (Weisskopf *et al*. 2011). Finally, Caballero-Mellado *et al*. (2004) found that *B. unamae* could be isolated from the rhizosphere of plants growing in soils with a pH range of 4.5-7.1, but not from soils with pH values higher than 7.5.

Burkholderia tolerance mechanisms relevant in acidic soils

The above mentioned reports show that *Burkholderia* species do not only survive in acidic soils but also actively grow in low pH environments. For that, species of *Burkholderia* must have developed tolerance mechanisms that allow them to cope directly with acidity but also with all other stresses that are present in such environments as a result of low pH. Even though the exact tolerance mechanisms in *Burkholderia* are yet to be resolved, few studies have suggested that low pH tolerance might be related to cell membrane rearrangements. In *B. cenocepacia*, hopanoid production has been shown to be essential for low-pH tolerance (Schmerk *et al.* 2011). That cell membrane components and cell shape are involved in nickel (Ni) and low pH tolerance was also suggested in a study on *B. cepacia* (Van Nostrand *et al.* 2008). Additionally to Ni tolerance, *Burkholderia* also acquired tolerance against multiple other heavy metals such as cadmium (Cd), zinc (Zn) and lead (Pb) (Lazzaro *et al.* 2008, Kuffner *et al.* 2010, Schwager *et al.* 2012, Jin *et al.* 2013) that become highly bioavailable in acidic soils and have negative effects on microbial growth and activities (Wang *et al.* 2007, Macomber and Hausinger 2011).

Rainfall can periodically create anoxic or at least micro-oxic conditions in the soil, and aerobic microbes need to develop mechanisms to overcome such periods. A recent publication showed that *Burkolderia* can tolerate micro-oxic conditions and survive in as little as 0.1% O_2, despite being described in the literature as obligate aerobes (Pessi *et al.* 2013).

Burkholderia species are well known as biofilm forming bacteria (Huber *et al.* 2001, Kim *et al.* 2013, Cuzzi *et al.* 2014). Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers (Costerton *et al.* 1999). Under laboratory conditions it has been show that this structural organization enables bacteria to tolerate many environmental stresses, which might be of great importance also in soils (Davey and O'Toole 2000, Templeton *et al.* 2001).

In view of all these above-mentioned tolerance mechanisms, members of the genus *Burkholderia* seem to be very well adapted to life in acidic soils.

Ecological functions of *Burkholderia* in the soil

As mentioned above, *Burkholderia* species can provide multiple ecological services. Studies on the active bacterial communities involved in important biogeochemical processes in a coniferous forest soil revealed that *Burkholderia* was one of the most important genera (Baldrian *et al*. 2012). McNamara and Leff (2004) studied the response of bacteria to dissolved organic matter (DOM) from decomposing maple leaves. Using artificial microbial assemblies of rhizosphere bacteria, among which *B. cepacia* was also included, the authors showed that the population size of this species rapidly increased during the first days of the decomposition, suggesting an important role in soil organic matter mineralization (McNamara and Leff 2004). These results were later confirmed by a study using a stable-isotope probing approach on natural soil microbial community (Štursová *et al*. 2012): *Burkholderia* were among the most abundant bacteria that accumulated C from cellulose and likely contributed to its decomposition. Furthermore, a recent study on lignocellulolytic bacteria in wet tropical forest soils from Puerto Rico showed that species belonging to the genus *Burkholderia* were among the dominant taxa contributing to the plant litter decomposition (both cellulose and lignin) (Woo *et al*. 2014).

In addition to their involvement in mineralization of plant material, a large number of *Burkholderia* species has been shown to actively degrade pollutants, toxic and mutagenic compounds, which they could even use as carbon source (Ortega-González *et al*. 2013, Dobslaw and Engesser 2014, Neumann *et al*. 2014, Pan *et al*. 2014).

Interactions of *Burkholderia* species with soil microbes

A possible survival strategy in acidic soils might be the close interaction with other soil microbes, as well as with higher organisms such as plants. The importance of interactions between plants and *Burkholderia* species has been widely documented (Moulin *et al*. 2001, Salles *et al*. 2004, Salles *et al*. 2006, Melkonian *et al*. 2014, Paungfoo-Lonhienne *et al*. 2014). However there are also numerous reports about interactions between *Burkholderia* and soil microbes, especially with fungi.

Fungi are ubiquitous organisms but are, like *Burkholderia*, mainly associated with the soil environment. Soil fungi represent a large proportion of the soil microbial

community and it has been shown that their growth and activity decrease with increasing pH (Rousk *et al*. 2009). Furthermore, when bacterial communities are eliminated through antibiotic treatment, fungi lose their preference for acidic pH, suggesting that there is a strong competition between these two groups of organisms in the soil (Rousk *et al*. 2009).

Competitive behavior between *Burkholderia* and fungi is well described and is linked to *Burkholderia*'s ability to produce antifungal compounds. Antifungal activity of *Burkholderia* towards fungi, mainly of phytophatogenic nature such as *Alternaria* sp. (Groenhagen *et al*. 2013), *Fusarium* sp. (Li *et al*. 2007), *Rhizoctonia* sp. (Quan *et al*. 2006), *Aspergillus* sp. (Palumbo *et al*. 2007) and *Phytophthora* sp. (Mao *et al*. 2006) has been shown for a number of *Burkholderia* species, in particular for those belonging to the BCC. Antifungal behavior derives from the production of numerous compounds such as pyrrolnitrin (Hammer *et al*. 1999), ornibactin (Lewenza and Sokol 2001), rhizoxin (Partida-Martinez and Hertweck 2007), burkholdin (Lin *et al*. 2012) and occidiofungin (Chen *et al*. 2013). However *Burkholderia* can also negatively affect fungi through the release of different volatiles (Kai *et al*. 2007, Groenhagen *et al*. 2013).

Such antagonistic interactions with phytopathogenic fungi have been so far only reported for the pathogenic group of *Burkholderia* (including BCC). However, for the non-pathogenic, environmental group of *Burkholderia*, opposite effects have been described. In contrast to life in rhizospheres or in plants, where nutrients are constantly provided, life in bulk soil is very difficult and nutrients are limited. Thus it is not surprising that symbiotic and mutualistic interactions have evolved between microbes. *B. rhizoxinica* was one of the first examples of bacterial endosymbionts described in fungi. This bacterium produces rhizoxin that is used by its host fungus, *Rhizopus* (Schmitt *et al*. 2008). Close association between *B. sordidicola* and the white-rot fungi *Phanerochaete* sp. (Lim *et al*. 2003) was described, where attempts to eliminate the bacteria from the fungus proved unsuccessful (Seigle-Murandi *et al*. 1996).

Recent research has focused on identifying specific ecological reasons for the establishment of these interactions. It has been shown that fungi provide hospitable conditions for *Burkholderia* in acidic soils by alleviating the low pH pressure (Nazir *et al*. 2010). Furthermore, secondary metabolites (e.g. glycerol), which are released by fungi, can be utilized by *Burkholderia* and can represent an important food source in a nutrient-depleted environment such as soil (Leveau and Preston 2008, Nazir *et al*. 2013). Moreover, *Burkholderia* take advantage of the extensive mycelial network that spreads in the soil by attaching to the hyphae and co-migrating with them during their growth (Warmink *et al*. 2011, Nazir *et al*. 2012).

Studies suggest that fungi may also benefit from such interactions. For example, *Burkholderia* species were enriched in the mycorrhizosphere of *Scleroderma citrium*, where they were involved in mineral weathering (Lepleux *et al*. 2012). This weathering activity provides fungi as well as plants with soluble minerals necessary for their growth (Uroz *et al*. 2007, Uroz *et al*. 2009).

Aims of the thesis

As described above, *Burkholderia* species represent an important part of the soil microbial communities, they provide a number of ecological benefits to the environment and they have the potential to be used in bioremediation processes as well as in agronomy as plant-growth promoters or biocontrol agents. This versatility and environmental relevance makes them interesting model organisms to study, and for putative application, a good understanding of their occurrence and ecological potential in the soil is necessary.

Literature suggests that *Burkholderia* might have a preference for low pH soils. However, no comprehensive study has yet been conducted on the whole genus, which would show whether observations from the literature are relevant for the whole genus or only for the described and isolated species. For this reason, we decided to analyze the biogeography of *Burkholderia* populations in the soil on two sampling scales (trans-continental and local scale) and assessed the putative role of pH in shaping their distribution in the soil. Additionally, we investigated the ability of *Burkholderia* to grow in different pH conditions and assessed their response to pH changes in natural soils.

In the second part of the thesis we investigated the nature of fungal-*Burkholderia* interactions, and in particular the changes occurring in the physiology of *Burkholderia* when co-cultivated with fungi.

The focus of the last project was the interactions of *Burkholderia* with plants and the role of oxalate in these interactions. We constructed a mutant, which was unable to degrade oxalate and monitored its capacity to colonize the roots of maize and lupin.

Part I: Biogeography of *Burkholderia* sp. in soil

Aims of the project

Literature suggests that *Burkholderia* species have a preference for acidic soils (Curtis *et al*. 2002, Belova *et al*. 2006, Kaestli *et al*. 2009). However, no study has yet investigated the distribution of the whole genus *Burkholderia*. Thus, the main objective of the project was to analyze the biogeography of *Burkholderia* across different soils, by investigating their abundance, diversity and community structure.

To assess the abundance of *Burkholderia* species in soils, a novel quantitative PCR protocol was needed, which would specifically target *Burkholderia* genes in environmental samples. In a recent pyrosequencing study, a primer pair targeting *Burkholderia* 16S rRNA gene was designed that might be a candidate for this qPCR protocol we needed to develop (Bergmark *et al*. 2012). However, optimization of a protocol based on this primer set would be necessary, since the results from this study showed high unspecificity of the primers towards the *Burkholderia* targets in environmental samples, even though *in silico* analysis guaranteed not only full coverage of the species across the genus *Burkholderia*, but also 100% specificity (Bergmark *et al*. 2012).

Burkholderia relative abundance and diversity were studied on two geographical scales, a trans-continental and a local scale, and correlated with environmental parameters described for the sampling locations. Soil DNA samples for the trans-continental study were provided by our collaborator Prof. Noah Fierer (University of Colorado at Boulder, USA) and covered a wide range of ecosystems. For the local scale, samples collected from an agricultural field in Scotland (UK) were selected due to the unique properties of the field, on which a pH gradient of pH 4.5–7.5 has been maintained for over 60 years. Furthermore, the direct effect of pH was also tested on pure cultures of a wide collection of *Burkholderia* strains in growth media with different pH, as well as in natural soils where initial pH was artificially altered.

Unpublished results

Design of a quantitative PCR (qPCR) protocol targeting the Burkholderia *16S rRNA gene*

To investigate the diversity of *Burkholderia* sp. and their community structure, we used previously described methods and primers targeting the 16S rRNA gene (Schönmann *et al*. 2009). Protocols to determine the abundance of few *Burkholderia* species were available (Wright *et al*. 2010, Price *et al*. 2012, Price *et al*. 2013). However, to follow the relative abundance of the complete genus *Burkholderia*, no protocol had yet been established. To this end, we had to develop a novel qPCR protocol that would specifically target *Burkholderia* genes and would cover the complete genus.

Primer selection and specificity assessment

Due to the extensive databases on the 16S rRNA gene, we decided to use this gene as molecular marker for our approach. We collected all sequences of 16S rRNA genes available at that time for the described *Burkholderia* species (n=72). To design the primers, we used sequence alignment tools such as MEGA5 (Tamura *et al*. 2011) and BioEdit (Hall 1999) as well as the ribosomal database project II (Cole *et al*. 2007). As additional criterion for the qPCR primer selection, the amplicon length had to be considered too, since it has a significant effect on qPCR efficiency.

With these criteria we developed one pair of primers that covered as much of the diversity as possible, was specifically targeting the *Burkholderia* genus and covered a region of 197 bp. Additionally, we also included primers that had been designed by Bergmark *et al*. (2012). This latter primer set was highly specific in *in silico* analyses, but the metagenomic results obtained from environmental samples showed very low specificity and primarily targeted *Pseudomonas* sp. 16S rRNA (Bergmark *et al*. 2012). A possible reason for this unspecific binding might be the pyrosequencing protocol and the low T_m (60°C).

To compare both primer pairs, they were first tested on single strains of *Burkholderia* and then on soils samples known to harbor *Burkholderia* species. Gradient PCR was

used to determine the optimal T_m for both primer pairs (Table 1). To determine the specificity of the method, DNA isolated from agricultural soils with different pH values (see below, Published results) was amplified with both primer pairs. The PCR products were subsequently excised from the agarose gels, purified, cloned using TOPO cloning kit (Invitrogen, USA) and finally plasmids containing PCR products were sequenced using the Sanger technique (80 plasmids per primer pair). Of both primer sets, only the set designed by Bergmark *et al*. (2012), used with an optimized T_m of 64°C, gave 100% specificity. The specificity of this primer pair was then further validated by sequencing 296 clones. After excluding the sequences from shorter or failed reads, 264 sequences remained and phylogenetic relatives were assigned using local blast and *Burkholderia* 16S rRNA database. This yielded 100% specificity for the *Burkholderia* 16S rRNA gene. Therefore, this primer pair was used to design the qPCR protocol.

Table 1. List of primers to investigate *Burkholderia* abundance in the soil. Two pairs of primers were tested, one pair designed *de novo* and the second designed by Bergmark *et al*. (2012)

Primer pair	Primer sequence	Length of PCR products	T_m after optimization	Reference	Unspecific phylogenetic targets from *in silico* analyses
BKH635F – BKH832R	5'-GCR RGC TAG AGT ATG GCA-3' and 5'-CAA CTA GTT GAC ATC GTT TAG GG-3'	197 bp	56°C	This study	Members of family *Rhodocyclaceae* (found in aquatic environments)
BKH812F – BKH1249R	5'-CCC TAA ACG ATG TCA ACT AGT TG-3' and 5'-ACC CTC TGT TCC GAC CAT-3'	437 bp	64°C	Bergmark *et al*. (2012)	none

Optimization of the qPCR method and measurement of Burkholderia sp. relative abundance in soils

To quantify *Burkholderia* 16S rRNA genes in soil samples, primers designed by Bergmark *et al*. (2012) were used with an optimized T_m in MicroAmp optical 96-well plates with automated ABI 7500 Fast sequence detector (Applied Biosystems (ABI), United Kingdom). Cycling conditions were 95°C for 5 min, 45 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 45 s, followed by collection of fluorescence data and melting curve analysis (64°C to 92°C). Using primer, $MgCl_2$ and template concentrations as described in the PCR protocol by Bergmark *et al*. (2012) resulted in low efficiency (~65%). However, an increase in efficiency was achieved by increasing concentrations of the template and of $MgCl_2$. Finally, each 25 µl reaction contained the following: 0.25 mg ml^{-1} bovine serum albumin (BSA), 0.7 µM primers, 12.5 µl of SYBR® Green PCR master mix (ABI, United Kingdom), 5 µl (approx. 10 ng/reaction) of nucleic acid template and additionally 2 mM $MgCl_2$. With these conditions, amplification efficiency was between 90% and 100%. Standard curves were generated from known amounts of linearized plasmid (pCR®2.1, Invitrogen) containing *B. gladioli* 16S rRNA gene fragment amplified by using primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Wilson *et al*. 1990) in a dilution series of 10^2 to 10^8 copies.

To obtain the relative abundance of *Burkholderia* 16S rRNA genes in the soils, we had to measure also the quantity of total bacterial 16S rRNA genes in the corresponding soil samples. To do this, we used the protocol described by Smith *et al*. (2006). Bacterial 16S rRNA genes were amplified with the primers 1369F and Prok1492R (Smith *et al*. 2006) and a dilution series (10^2 to 10^9) of linearized plasmid as described above was used. Cycling conditions were 95°C for 5 min, 40 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s, followed by collection of fluorescence data and melting curve analysis (64 to 92°C). PCR was performed using an ABI 7500 Fast sequence detector as previously mentioned and each 25 µl reaction contained the following: 0.25 mg ml^{-1} bovine serum albumin (BSA), 0.8 µM bacterial 16S rRNA primers, 12.5 µl of SYBR® Green PCR master mix (ABI, Warrington, United Kingdom) and 5 µl (approx. 10 ng/reaction) of nucleic acid template.

Each plate included triplicate reactions per DNA sample and the appropriate set of standards. After the DNA amplification cycles, melting curve analysis was performed to confirm that the obtained signals were caused by the specific amplicon and additionally PCR products were visualized by standard 1% agarose gel electrophoresis. The C_T values for each PCR reaction were automatically calculated and analyzed by the ABI prism sequence detection systems software (version 2.0). A standard curve was obtained by plotting the C_T values as a function of the log-transformed copy numbers of linearized plasmid.

Effect of pH change on the abundance of the genus **Burkholderia**

In our biogeography study (see below, Published results (Stopnisek *et al*. 2014)), we have shown that pH has a significant effect on the relative abundance of *Burkholderia* in soils at a trans-continental scale, and an even higher effect at a local scale. To investigate in more detail the importance of soil pH for their abundance, we developed a microcosm system using the low pH and the high pH soils collected from the local scale, i.e. from an agricultural field with a pH gradient (Stopnisek *et al*. 2014). The original pH of the soil was then either increased or decreased by addition of $Ca(OH)_2$ or H_2SO_4 respectively. The C/N ratio and other soil chemical parameters such as Al, Ca, Fe, K, Mg, Mn, Na, Pb and Zn, were measured.

Material and methods

Construction of microcosms

Soil samples were collected in October 2011 from an agricultural field with original pH of 4.5 (±0.03) and 7.5 (±0.02). The soils were sampled in biological triplicates from the upper 20 cm soil layer, homogenized and stored at 4°C prior usage. The effect of pH changes on *Burkholderia* relative abundance was studied in a microcosm study where soil pH was adjusted from acidic to moderately acidic and from alkaline to acidic pH values. Adjustment of pH was achieved by using solutions of $Ca(OH)_2$ and H_2SO_4 to reach a final pH of 6.25 (±0.02) and 4.67 (±0.04), for the acidic and alkaline soil respectively. The treated soils were pre-incubated for 24 h prior to constructing the microcosms. Triplicate microcosms consisted of 10 g of wet pre-incubated soil and were incubated for 1, 7, 14, 21 and 28 days at room temperature. Microcosms were opened every 3 days to maintain oxic conditions and a water holding capacity (WHC) of 60% was maintained by addition of distilled water as determined by weight loss. After each time point 4 g of soil was removed from each microcosm and immediately frozen at -20°C for nucleic acid isolation and molecular analyses. Total nucleic acids were extracted in triplicates from 0.5 g of soil as described by Griffiths *et al*. (2000) with some modifications (Nicol *et al*. 2005). Technical triplicates of the biological replicates were pooled and used for further molecular analyses. The remaining 6 g of soil was used for determination of pH and soil chemistry.

Measurements of soil properties

pH was measured in deionized water using a ratio of 1:2 soil:water (w/v), shaking for 30 min and settling for 30 min before measurement. To test if altering soil pH also changed soil chemical properties, we determined selected soil chemical parameters before treatment and after 14 and 21 days of incubation. For exchangeable metal cations (Al, Ca, Fe, K, Mg, Mn, Na, Pb and Zn), soil samples were extracted with 1 M NH_4Cl, filtered and measured using ICP-AES. Total carbon (C) and nitrogen (N) content in soils were measured using 25 mg dried and finely ground (disk mill) soil weighed into tin capsules introduced into a Flash elemental analyser (Thermo Fisher Scientific, Switzerland) operated with He as a carrier gas. The samples were combusted in the presence of O_2 in an oxidation column at 1030°C and the combustion gases passed through a reduction column (650°C). The N_2 and CO_2 gases produced were separated chromatographically and the amount determined with a thermal conductivity detector. The contents were calibrated by bracketing with a standard soil with known C and N concentrations.

Measuring relative abundance of *Burkholderia* in microcosm soils

The relative abundance of *Burkholderia* 16S rRNA genes was determined by our designed qPCR protocol. DNA isolated from the microcosm soils was diluted 50x and 100x for the quantification of *Burkholderia* and of bacteria, respectively. All PCR products were checked for their specificity (product size) on 1% agarose gel electrophoresis. There was a linear relationship between the log of the plasmid DNA copy number and the C_T values across the specified concentration range (r^2 values between 0.995 and 0.999) and a slope of 3.55 to 3.32 and 3.50 to 3.32 (data not shown) for *Burkholderia* 16S rRNA assay and bacteria 16S rRNA assay, indicating a high amplification efficiency between 91% to 100% and 93% to 100%, respectively. The data were presented as ratio between *Burkholderia* and bacteria copy numbers g^{-1} dry $soil^{-1}$.

Results and discussion

In control microcosms, the relative abundance of *Burkholderia* remained fairly constant over the incubation time (28 days) (Figure 2). Treating the low pH soil (original pH 4.5±0.03), with 5.4 M $Ca(OH)_2$ led to an increase in pH, with a stable final pH of 6.25 (±0.02). Pre-incubation of the soil for one day after the alkaline treatment resulted in a transient increase in relative abundance of *Burkholderia* ($P < 0.05$), possibly due to a decrease in relative abundance of other acidophilic taxa. *Burkholderia* relative abundance then significantly decreased over the incubation time (Figure 2A).

In the soil with an original pH of 7.5 (±0.02), addition of 7.2 M H_2SO_4 resulted in a decrease in pH, reaching a stable final pH of 4.67 (±0.04). The pH decrease led to more than four-fold increase in the relative abundance of *Burkholderia*, from 0.06 % at the start of the experiment to 0.27 % at day 28 ($P < 0.05$) (Figure 2B).

The C/N ratios in the microcosms were similar for both soils (between 15.4 and 14.7) and did not change over incubation time or between treatments (Figure 3), clearly demonstrating that pH itself, and not C/N, which was correlated with pH in our biogeography study (Stopnisek *et al*. 2014), was responsible for the changes in *Burkholderia* relative abundance in these soils.

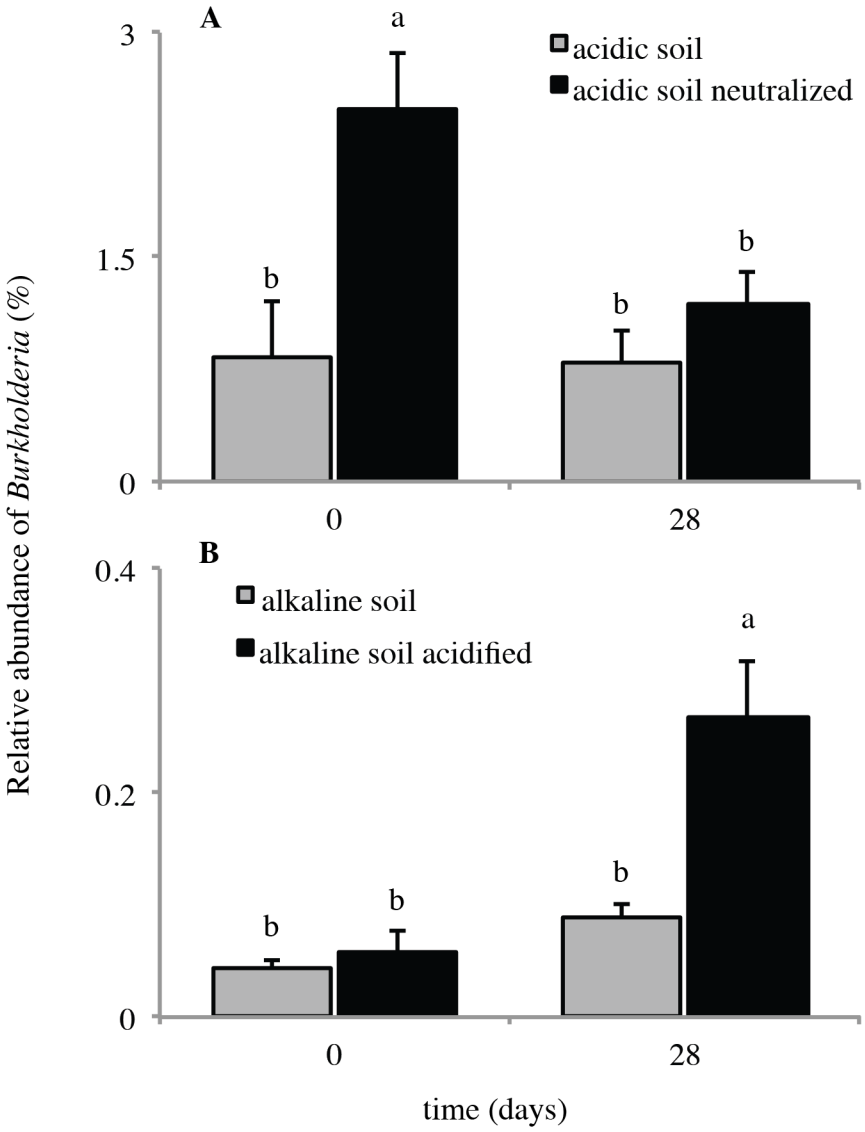

Figure 2. Relative abundance of *Burkholderia* 16S rRNA genes in a manipulative microcosm study. (A) Relative abundance of *Burkholderia* in microcosms with acidic soil (pH 4.5). (B) Relative abundance of *Burkholderia* in microcosms with alkaline soil (pH 7.5). Grey bars represent the control microcosms and black bars represent the pH-adjusted microcosms. Error bars represent SD. The same letters above the bars indicate absence of significant differences between treatments and time of incubation as calculated by pairwise t test ($P < 0.05$, n=3).

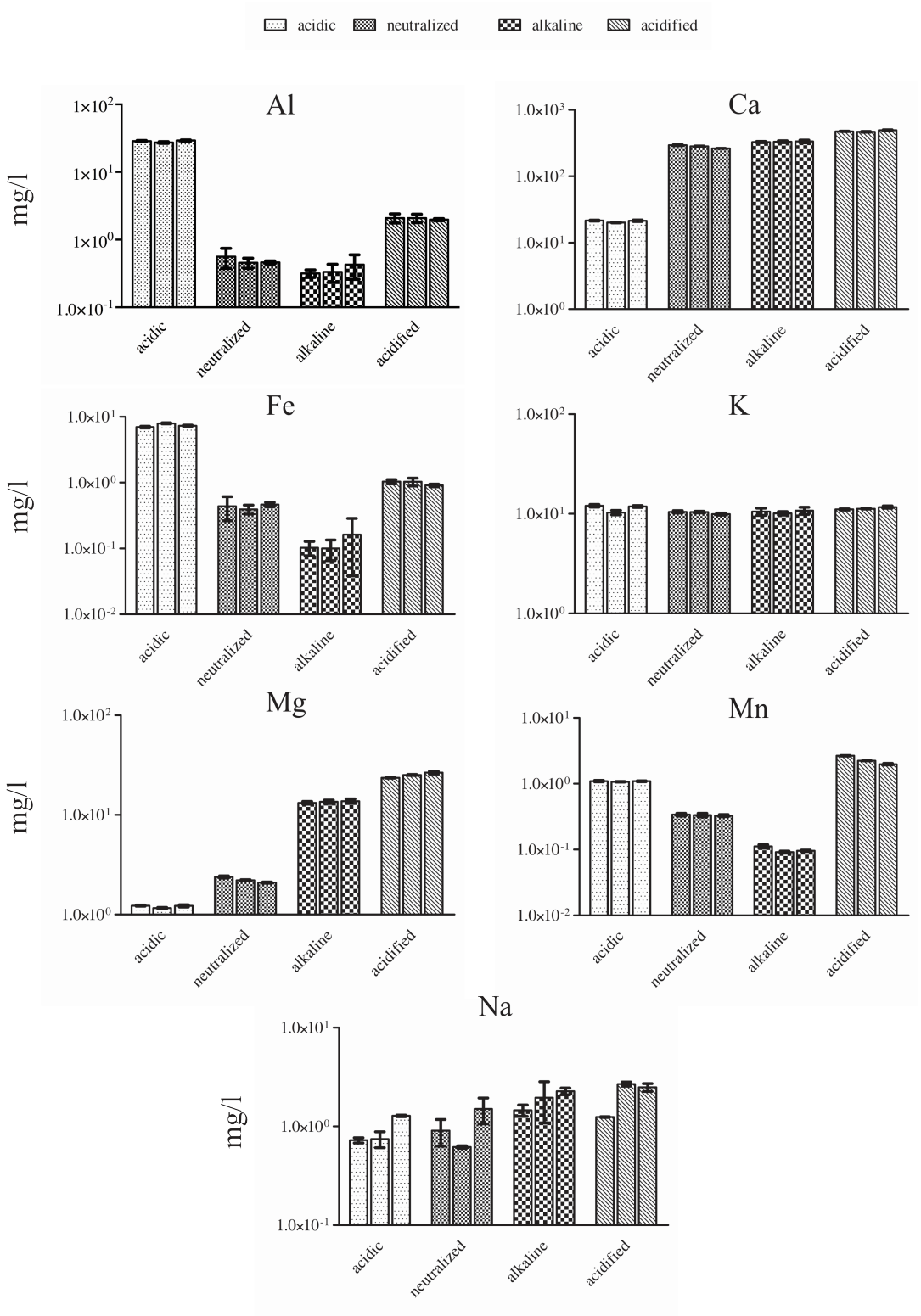

Figure 3: Soil chemical analysis. (continue on next page)

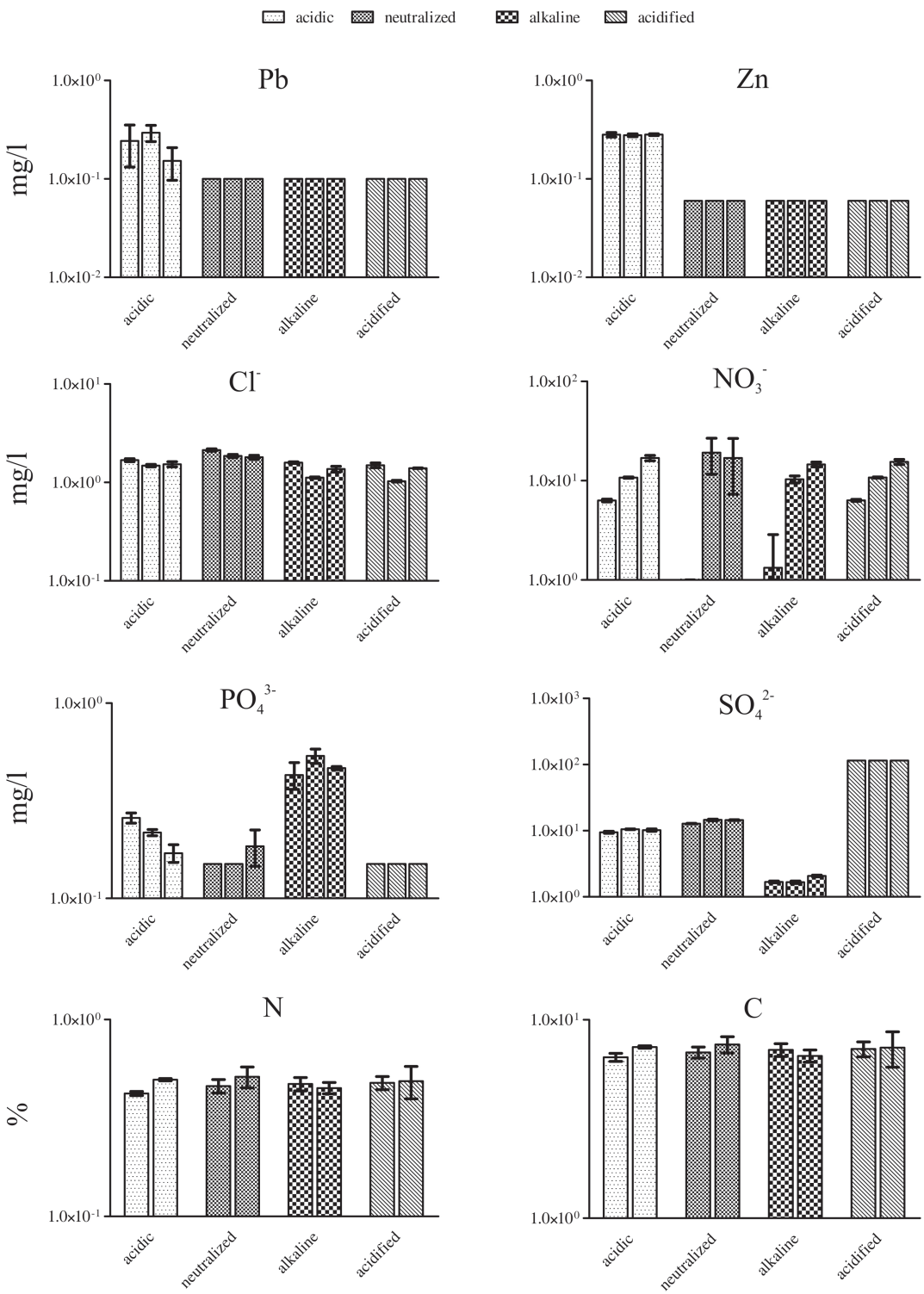

Figure 3 (cont.): Soil chemical analysis. Bar charts represent measured chemicals in soil microcosms with native (acidic and alkaline) and pH adjusted (neutralized and acidified) sols. Bars represent time of sampling: 0, 14 and 28 per each condition except of N and C where only day 0 and 28 are represented. Error bars represent standard errors. PO_4^{3-}. Unites of y-axis are represented on the left side of the bar charts (log scale).

environmental microbiology

Environmental Microbiology (2014) **16**(6), 1503–1512 doi:10.1111/1462-2920.12211

Genus-wide acid tolerance accounts for the biogeographical distribution of soil *Burkholderia* populations

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Summary

Bacteria belonging to the genus *Burkholderia* are highly versatile with respect to their ecological niches and lifestyles, ranging from nodulating tropical plants to causing melioidosis and fatal infections in cystic fibrosis patients. Despite the clinical importance and agronomical relevance of *Burkholderia* species, information about the factors influencing their occurrence, abundance and diversity in the environment is scarce. Recent findings have demonstrated that pH is the main predictor of soil bacterial diversity and community structure, with the highest diversity observed in neutral pH soils. As many *Burkholderia* species have been isolated from low pH environments, we hypothesized that acid tolerance may be a general feature of this genus, and pH a good predictor of their occurrence in soils. Using a combination of environmental surveys at trans-continental and local scales, as well as *in vitro* assays, we show that, unlike most bacteria, *Burkholderia* species have a competitive advantage in acidic soils, but are outcompeted in alkaline soils. Physiological assays and diversity analysis based on 16S rRNA clone libraries demonstrate that pH tolerance is a general phenotypic trait of the genus *Burkholderia*. Our results provide a basis for building a predictive understanding of the biogeographical patterns exhibited by *Burkholderia* sp.

Received 17 May, 2013; revised 4 July, 2013; accepted 10 July, 2013.
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Introduction

The genus *Burkholderia*, which belongs to the β-Proteobacteria class, currently comprises more than 60 species that are widely distributed and frequently isolated from a large range of natural and clinical environments (Compant *et al.*, 2008). The genus *Burkholderia* can be divided phylogenetically into two main clusters: the first one consists mainly of human, animal and plant pathogens, e.g. the *Burkholderia cepacia* complex (Bcc) and the rice pathogen *B. glumae*. However, it is important to note that some strains belonging to Bcc, such as *B. ambifaria* or *B. lata*, also show plant growth-promoting abilities as well as biocontrol activities against phytopathogenic fungi. The other cluster consists mainly of plant-beneficial-environmental (PBE) *Burkholderia* species (Suárez-Moreno *et al.*, 2012). The members of the first cluster have been extensively studied because of their medical importance, but recently the PBE cluster has been the focus of research efforts with the discovery that various species of this cluster are able to fix nitrogen (Estrada-De Los Santos *et al.*, 2001; Martínez-Aguilar *et al.*, 2008) and to nodulate legumes (Moulin *et al.*, 2001). *Burkholderia* from the PBE cluster have been mainly isolated from plant rhizosphere, but they are also frequently detected in sediment and bulk soil (Salles *et al.*, 2002; Lazzaro *et al.*, 2008; Lim *et al.*, 2008; Lepleux *et al.*, 2012; Štursová *et al.*, 2012). In addition to their nitrogen-fixing and nodulating abilities, their versatile metabolism also enables them to survive in harsh conditions, such as nutrient-limited or polluted environments, and to degrade recalcitrant compounds (Pérez-Pantoja *et al.*, 2012). As such, they have been suggested as good candidates for use in biotechnology, e.g. for bio- or phytoremediation, biocontrol and biofertilization. Despite the high relevance of the *Burkholderia* genus for human health, agronomy and biotechnology, surprisingly little is known about the factors underlying their geographical distribution.

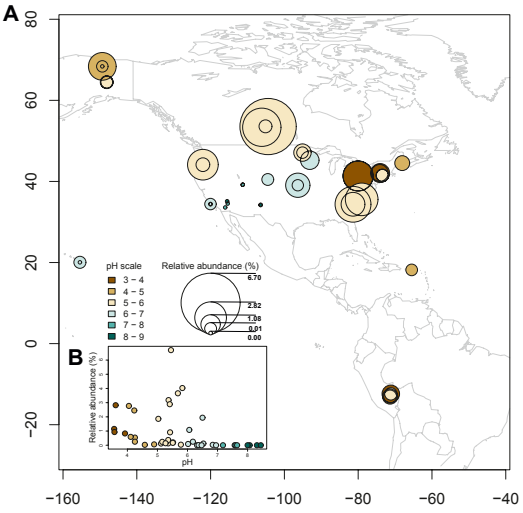

Fig. 1. Relative abundance of *Burkholderia* 16S rRNA genes in 44 soils.
A. Representation of *Burkholderia* 16S rRNA gene relative abundance at the different sites as assessed by qPCR. Relative abundance is represented by the circle size; the colour indicates the pH of the sampled soils.
B. Influence of pH on the relative abundance of *Burkholderia* 16S rRNA genes. Circles represent the average of three replicates for each soil sample.

Soil pH has frequently been shown to be the main predictor of overall soil bacterial community composition, diversity and the relative abundance of many individual taxa (Tiedje *et al*., 1999; Fierer and Jackson, 2006; Rousk *et al*., 2010; Griffiths *et al*., 2011). Although it is not known if *Burkholderia* distributions are related to soil pH, most studies that have reported their presence in soil were investigating acidic environments (Trân Van *et al*., 2000; Curtis *et al*., 2002; Salles *et al*., 2002; 2004; Belova *et al*., 2006; Garau *et al*., 2009; Aizawa *et al*., 2010). For instance, *Burkholderia unamae* could only be isolated from the rhizosphere of plants growing in soils, with a pH ranging from 4.5 to 7.1, but not from soils with a pH higher than 7.5 (Caballero-Mellado *et al*., 2004). Likewise, a survey of over 800 Australian soil samples revealed that *B. pseudomallei* was specifically associated with low pH soils, but not recovered from higher pH soils (Kaestli *et al*., 2009). *Burkholderia* species have also been isolated from acidic *Sphagnum* peat bogs (Belova *et al*., 2006; Opelt *et al*., 2007a; 2007b), from root tissues of the highly acidifying cluster rooted *Lupinus albus* (Weisskopf *et al*., 2011) or from soils as acidic as pH 2.9 (Curtis *et al*., 2002). To the best of our knowledge, only one study reported isolation of *Burkholderia* strains from an alkaline environment (Estrada-de los Santos *et al*., 2011). While the pH of the rhizosphere soil investigated in this study was high (8.7), the isolated strains were all able to grow at low pH (4.5). These reports provide anecdotal evidence that *Burkholderia* might be tolerant to low pH conditions, which enables members of this genus to thrive in niches where others would be inhibited. We, therefore, hypothesized (i) that low pH tolerance is an intrinsic phenotypic trait of the *Burkholderia* genus, and (ii) that the relative abundance and diversity of *Burkholderia* populations are highest in low pH soils, with the biogeography of *Burkholderia* predictable from soil pH. To test these hypotheses, we developed a novel quantitative polymerase chain reaction (qPCR) protocol to analyze the relative abundance of *Burkholderia* populations in soils at a trans-continental and a local scale. Intrageneric diversity and community structure were determined by 16S rRNA-based clone libraries constructed from a selected subset of the trans-continental scale soil samples. In addition, *in vitro* physiological assays were used to test the direct effects of pH on *Burkholderia* species.

Results

Low pH tolerance, a genus-wide property of Burkholderia *that largely accounts for its relative abundance in soils*

To test whether the occurrence of *Burkholderia* species in acidic environments reflects an intrinsic capacity of this genus to tolerate low pH conditions, we tested the ability of 68 strains of *Burkholderia* belonging to 31 different species to grow at a pH range of 3.5–8. All *Burkholderia* strains that were tested in physiological assays grew in pH as low as pH 4.5. Out of 68 tested strains (31 different species), 32 (18 species) were growing also at pH 4 and 15 (8 species) even at pH 3.5, but no species-specific tolerance could be observed under such conditions (Table S1).

Based on this result, we hypothesized that members of the *Burkholderia* genus would be favoured in low pH environments. We tested this hypothesis by analyzing the relative abundance of soil *Burkholderia* in a trans-continental sample collection of 44 soils from a broad array of ecosystem types that represent a wide range of soil and site characteristics (Table S2). The relative abundance of *Burkholderia* species greatly varied between these different soils sampled across North and South America (Fig. 1A). Highest relative abundance was observed in moderately acidic soils (pH 5–pH 6), where up to 6.7% of the total bacterial population was represented by *Burkholderia* species. It is worthwhile to notice that within this pH range a large variability in *Burkholderia* relative abundance was observed (standard deviation = ± 2.02), spanning from 0.04% to 6.25%, whereas in more acidic soils (pH <4) relative abundances were approximately 1% or higher. While high relative abun-

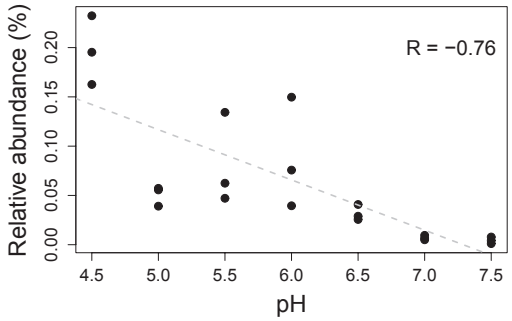

Fig. 2. Relative abundance of *Burkholderia* 16S rRNA genes along a local pH gradient in an agricultural field in Scotland. Relative abundance was quantified by qPCR in three soil samples for each pH along the pH gradient. Abundance of *Burkholderia* is strongly and negatively correlated with pH (Pearson's product-moment correlation: R = –0.76, $P < 0.001$).

dance of *Burkholderia* 16S rRNA gene copy numbers was detected in soils with a pH lower than 7, the relative abundance was under the detection limit of our qPCR method (less than 100 copies reaction^{-1}) in neutral and alkaline soils (Fig. 1B). As expected, pH was a significant factor predicting *Burkholderia* relative abundance ($P = 0.03$, R = –0.33), although the correlation was weak, probably due to the high variability of *Burkholderia* relative abundance in low pH soils. Moreover, pH is not the only variable that changes across the soils analyzed, and pH often correlates with other soil and site characteristics. The C/N ratio showed the best correlation ($P = 0.0005$, R = 0.50) with *Burkholderia* relative abundance, but since pH and C/N ratio correlate ($P = 0.003$, R = –0.4380), we tested our hypothesis in a different experimental set-up, in which the effect of pH could be discriminated from that of C/N ratio. To this end, we analyzed the relative abundance of *Burkholderia* in an agricultural field with a pH gradient of 4.5–7.5 but a constant C/N ratio. Relative abundance of *Burkholderia* 16S rRNA was lower in this soil than in soils collected across North and South America. Highest relative abundance was detected at pH 4.5 (0.23–0.16%), and an almost linear decrease with increasing pH was observed, reaching 0.01–0.008% of *Burkholderia* 16S rRNA relative abundance in soil of pH 7.5 ($P < 0.0001$, R = –0.76) (Fig. 2).

Intrageneric diversity of Burkholderia *soil populations does not depend on pH*

To analyze the intrageneric diversity of soil *Burkholderia* populations and to determine whether some groups showed any pH preference, 14 sites varying in pH (from 3.5 to 6.8), C/N ratio, location and relative abundance of *Burkholderia* 16S rRNA genes were selected from the trans-continental scale sampling set for phylogenetic analyses. Clone libraries targeting the 16S rRNA gene were constructed for each of the selected sites, and a total of 675 sequences (590 bp) were obtained, corresponding to 123 operational taxonomical units (OTUs) at a 98% identity threshold between *Burkholderia* 16S rRNA gene sequences (Fig. 3). Diversity and richness of the soil *Burkholderia* communities were highly variable between the sites, e.g. only one phylotype was found in KP2 or PE3, but CL4 and PE5 harboured 33 and 25 phylotypes respectively (Fig. 3). However, there was no significant correlation between pH and *Burkholderia* diversity. To test whether pH or any other of the described environmental parameters could influence *Burkholderia* community composition, a Mantel test was performed (see Table 1). Our data showed that pH had no correlation with community structure ($r_M = 0.110$, $P = 0.204$), while site elevation and spatial parameters did significantly positively correlate with *Burkholderia* communities ($r_M = 0.39$, $P = 0.002$ and $r_M = 0.38$, $P = 0.038$ respectively). *Burkholderia* community structure was also marginally influenced ($P < 0.1$) by climatic factors and soil chemistry ($r_M = 0.295$, $P = 0.077$ and $r_M = 0.260$, $P = 0.094$ respectively). These results indicate that low pH would generally affect the relative abundance of the *Burkholderia* genus, but not the relative abundances of individual species within this genus, which is in line with our observations that low pH tolerance is a genus-wide feature of *Burkholderia* sp. (Table S1).

Burkholderia glathei*: a major and widespread soil inhabitant*

Within the entire sequence set, those closely related to *B. glathei* were by far the most abundant and most widely distributed of all (approx. 40% of sequences). These sequences comprised four OTUs, which contained 190, 33, 32 and 12 sequences respectively (Fig. 3). The most widespread and abundant OTU (190 sequences) was present at nine sites out of 14. Interestingly OTU 3, the next most abundant OTU of this *B. glathei* group, was present only at one site (HI3) and represented all of the 33 sequences collected at this site. The next most abundant OTU beside the *B. glathei* group was most closely related to *B. terricola* (53 sequences). Unlike the *B. glathei* group, however, this OTU was only found in one site (KP2), where it was the only *Burkholderia* representative. Sequences closely related to *B. phenazinium*, *B. fungorum* and *B. terrae* were very abundant as well, while other OTUs were represented by less than 20 sequences, and a high proportion (103 OTUs) consisted of less than five sequences (Table S3). Despite the high diversity of *Burkholderia* in some of our soil samples,

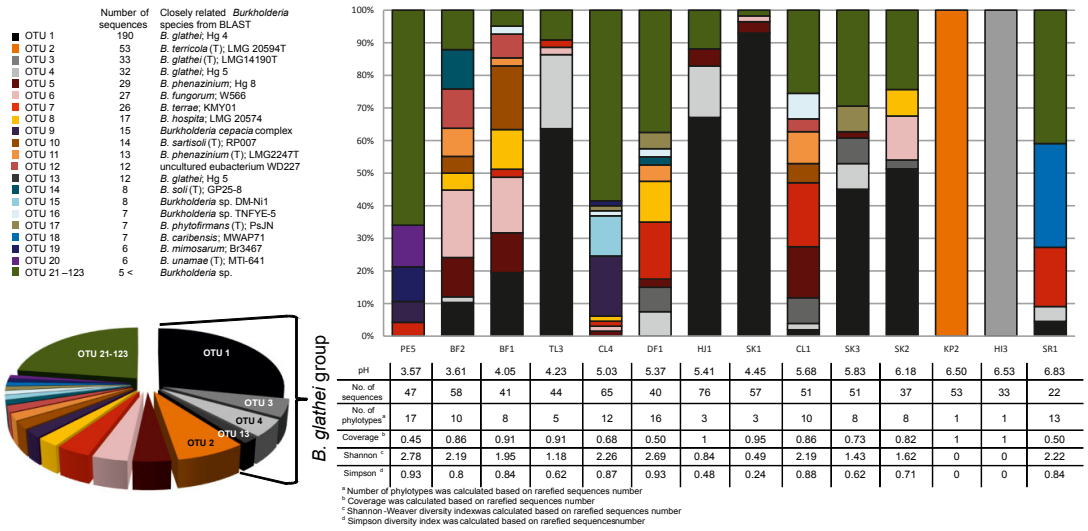

Fig. 3. Intrageneric diversity of soil *Burkholderia*. Pie chart represents the total number of OTUs obtained from 14 sites. Pieces represent OTUs that contain more than five sequences and are ordered by size (number of sequences). Dark green represents OTUs that contain less than five sequences. The largest group of sequences, which is represented by strains of *B. glathei*, is highlighted. The bar chart represents the relative abundance of OTUs per site, ordered by pH. The table contains the diversity indices calculated using rarefied sequence number (22 sequences) per site. All indices were calculated using 98% identity between sequences.

rarefaction curves reached a plateau in almost all soils analyzed (Fig. S1). The least diverse sites were HI3, KP2 and SK1 having less than four OTUs (Fig. 3). In all except for KP2, the majority of sequences were affiliated with *B. glathei* (100% at site HI3 and 93% at site SK1), suggesting a preeminent role of this species in the soil.

Discussion

Low pH tolerance is a general property of the genus Burkholderia

All *Burkholderia* strains that were tested on minimal medium showed tolerance to acid pH (pH 4.5). Similar results were obtained by Estrada-de los Santos and colleagues (2011), who reported that most of the 43 tested *Burkholderia* species were able to grow in a pH range of 5–11, although the medium used in their study was neither buffered nor was growth quantitated. Our results are in agreement with previous reports (Belova *et al.*, 2006; Aizawa *et al.*, 2010; 2011; Schmerk *et al.*, 2011) and suggest that *Burkholderia* are acidotolerant rather than acidophilic. The unveiling of this genus-wide acid tolerance allows conclusions on the lifestyle and environmental adaptation of these bacteria, and also offers new possibilities to select or enrich *Burkholderia* isolates from complex environments.

Burkholderia *are relatively more abundant in low pH soils*

Our results demonstrated a negative correlation between pH and relative abundance of *Burkholderia* 16S rRNA

Table 1. Relationship of *Burkholderia* community structure to combined and individual environmental parameters revealed by Mantel test.

Environmental parameters	r_M	*P*
Soil chemistry	0.260	**0.094**
pH	0.110	0.204
C/N ratio	–0.197	0.916
% organic C	0.226	0.126
Climatic	0.295	**0.077**
MAT	0.273	**0.042***
MAP	0.141	0.231
Soil	0.176	0.170
% silt and clay	0.289	**0.030***
Depth of O horizon	–0.119	0.666
SMD	0.125	0.236
Biological	0.227	0.125
C mineralization rate	0.226	0.134
Spatial (*longitude, latitude*)	0.379	**0.038***
Site elevation	0.387	**0.002***

Parameters highlighted in bold represent combined matrices that were used and included factors highlighted in italic (used also separately). Bold values represent significant *P* values (< 0.1). *P* values < 0.05 are indicated in bold and with an asterisk. r_M, Mantel's correlation coefficient.

genes: *Burkholderia* relative abundance was higher in acidic than in neutral soils and was absent or under the detection limit in alkaline soils. The highest relative abundance was observed in moderately acidic soils (pH 5–6), where *Burkholderia* represented 6.25% of the total bacterial 16S gene copies. Similar relative abundances of *Burkholderia* were previously observed in pyrosequencing studies investigating acidic bulk soils (1.2%), while abundances increased in the rhizosphere (1.96–3.08%) and even more in the mycorrhizosphere environments (3.30–8.33%) (Uroz *et al*., 2010; 2012). C/N ratio was another environmental parameter that significantly influenced the relative abundance of *Burkholderia* populations in soils, but this parameter was also, as often observed, strongly correlated with pH (Kemmitt *et al*., 2006). For this reason, we conducted a local-scale study on an agricultural field with a pH gradient where the C/N ratio is fairly constant and where the aboveground plant community is the same. Our data showed that the effect of pH on *Burkholderia* relative abundance was even stronger than what was observed on the trans-continental scale, with an almost linear decrease with increasing pH, which strongly suggests that pH, rather than C/N ratio, influences the distribution of *Burkholderia* populations in soil.

Intrageneric diversity of Burkholderia *soil populations does not depend on pH*

Previous studies investigating the phylogeny of acidobacteria have shown that their relative abundance and intrageneric diversity are higher in low pH soils. Interestingly, certain subgroups within this genus were identified, which were only found in neutral or even in alkaline soils (Lauber *et al*., 2008; Jones *et al*., 2009; Griffiths *et al*., 2011). Since our results of *Burkholderia* relative abundance are similar to the trends observed for acidobacteria, we investigated whether certain *Burkholderia* lineages would have a preference for soils with a particular pH. However, our diversity analysis showed no correlation between pH and community composition within the genus. While the intrageneric diversity varied greatly between the samples, no OTU was found that was specifically enriched in highly or moderately acidic soils. This is in line with our *in vitro* low pH tolerance assays, which suggested that pH tolerance is a general feature of the genus *Burkholderia*. Interestingly, pathogenic species, such as *B. pseudomallei* or *B. mallei*, or opportunistic pathogens, such as members of the Bcc, were very rarely detected in our soil survey, indicating that while they have been reported to be major inhabitants of maize (Bevivino *et al*., 2011) or sugar cane (Castro-González *et al*., 2011) rhizospheres, they are not commonly present in nutrient-limited bulk soil. In contrast, we observed very high relative abundance of *B. glathei*, which was in this study by far the most abundant and widespread OTU (Fig. 4). *B. glathei* has been previously shown to be widely distributed across soils and rhizospheres (Belova *et al*., 2006; Uroz *et al*., 2007; 2012). Here, we show that members of this species are not only very abundant in different soil and ecosystem types but are also extremely widespread over diverse geographical sites. This suggests that *B. glathei* is a preeminent soil inhabitant, which is particularly well adapted to this type of environment, although the specific functions responsible for the success of this species in soil remain undetermined. In addition to site descriptors analyzed in this study, biological factors may have an important role in shaping *Burkholderia* community composition and might be responsible for the highly variable intrageneric diversity observed in the selected soil samples. A good example of such biological factors is the symbiotic association between nitrogen-fixing *Burkholderia* species and plants. For example, *Burkholderia mimosarum* is capable of nodulating *Mimosa* plants, and is therefore only found in areas where the plants are endemic, such as tropical regions of South-eastern Asia and South America (Chen *et al*., 2006; Elliott *et al*., 2009). In line with this, we detected *B. mimosarum* only at site PE5, which is located in Peruvian Amazonas.

In summary, this study showed that low pH tolerance is a genus-wide feature of *Burkholderia* species. This explains their presence in acidic soils but not their absence from higher pH environments, especially considering that under laboratory conditions, the majority of *Burkholderia* strains are able to grow in neutral or even alkaline culture media. This suggests that *Burkholderia* have developed pH tolerance mechanisms that enable them to survive and thrive in environmental niches where many other taxa are inhibited, while they are outcompeted by faster growing microorganisms in less harsh conditions. Acid tolerance is a prerequisite for occurrence in low pH soils, but it is tempting to postulate that the preference of *Burkholderia* for such niches is not only the consequence of the ability to tolerate acidity, but the result of a multifaceted strategy involving both tolerance to abiotic stress factors (such as higher toxicity of heavy metals) and to biological constraints (e.g. the predominance of fungi) inherent to such environments.

Experimental procedures

Testing growth of Burkholderia *strains* in vitro *under different pH conditions*

To study the effect of pH on *Burkholderia* growth, an *in vitro* approach was used. To this end, 68 *Burkholderia* strains from different isolation origins were used (Table S1). Before spotting 20 μl aliquots of each culture on growth medium, over-

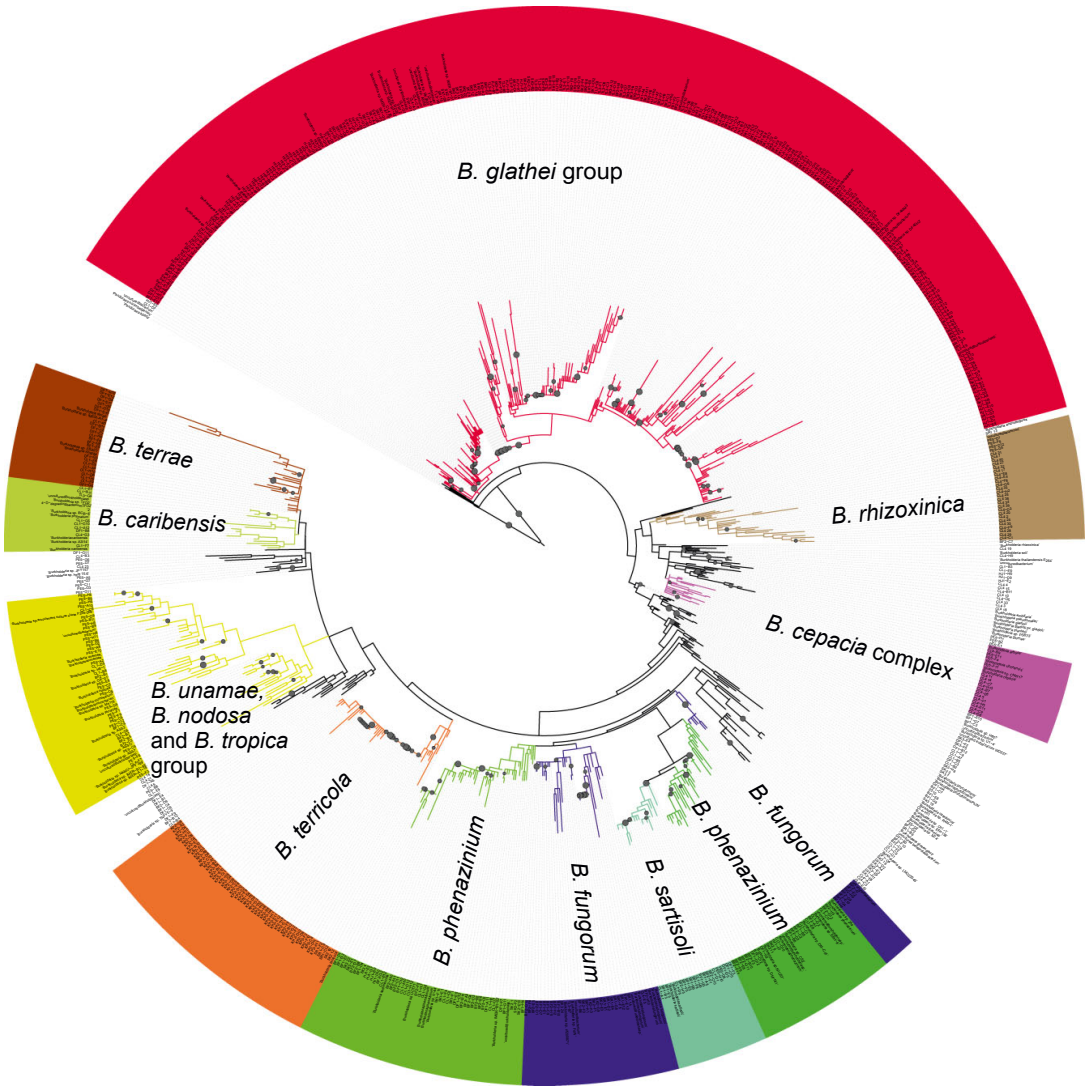

Fig. 4. Phylogenetic analysis of *Burkholderia* 16S rRNA gene sequences from 14 different sites. 675 sequences (590 bp) were aligned with additional reference sequences using ARB. Phylogeny was constructed using a maximum likelihood-based method. Bootstrap values ($n = 1000$) >50% are shown as circles. Colours indicate the affiliation with a given species.

night liquid cultures were washed and resuspended in saline buffer to optical density (OD_{600}) of 1. As growth medium, AB minimal medium (Clark and Maaløe, 1967) supplemented with glucose and agar was used, and the pH of the medium was adjusted to obtain a pH gradient of pH 4–7 in 0.5 unit steps, with an additional medium of pH 8. To test growth under more acidic conditions, liquid AB medium supplemented with glucose was used, adjusted to pH 3, pH 3.5 and pH 4. Liquid AB medium with pH 4 was used as a control to test if the growth patterns were the same between solid and liquid conditions. Media were buffered with 0.1 M potassium hydrogen phthalate ($C_8H_5KO_4$, $pK_a = 5.4$) and 0.1 M HCl for pH 3, 0.1 M $C_8H_5KO_4$ and water for pH 4, 0.1 M NaOH and 0.1 M $C_8H_5KO_4$ for pH 5–6, and for media higher than pH 7, 50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was added. To detect changes in pH caused by bacterial growth, resazurin was added as a pH indicator. Plates were incubated for 4 days at 30°C. Growth was assessed by inspecting the plates for the formation of colonies.

Soil sampling and DNA extraction

The dataset consisted of 44 soil samples distributed across North and South America. The collected soils came from a broad range of ecosystems, climates and soil types (Table S2). Soil collection protocol and methods for edaphic and environmental properties have been described previously (Fierer and Jackson, 2006; Bates *et al.*, 2011). In addition, seven soil samples were collected in March 2011 from an agricultural field divided into several plots with a pH gradient of 4.5–7.5, which has been maintained since 1961 by the addition of either lime or aluminium sulphate, and where plots undergo an 8-year crop rotation cycle (Scottish Agricultural College, Aberdeen, Scotland; grid reference NJ872104). Detailed soil characteristics are provided by Kemp and colleagues (1992). The soils were sampled in triplicates from the upper 20 cm soil layer, homogenized and stored at 4°C prior usage. Total nucleic acids were extracted from 0.5 g of soil, as described by Griffiths and colleagues (2000) with some modifications (Nicol *et al.*, 2005). pH was measured in deionized water using a ratio of 1:2 soil : water (w/v), shaking for 30 min and settling for 30 min before measurement.

qPCR

To quantify *Burkholderia* 16S rRNA genes in soil samples, primers BKH812F (5′-CCC TAA ACG ATG TCA ACT AGT TG-3′) and BKH1249R (5′-ACC CTC TGT TCC GAC CAT-3′) (Bergmark *et al.*, 2012) were used. In their original publication, Bergmark and colleagues (2012) observed that the designed primers were not specific, suggesting that the most likely explanation for lack of specificity was that the Tm value they used was too low. We, therefore, tested both primers for their specificity using higher Tm values on DNA isolated from soils with different pH. An annealing temperature of 64°C was found to efficiently amplify *Burkholderia* 16S rRNA genes with 100% specificity. This was tested by sequencing 270 clones containing PCR products from soils with three different pH. For both bacterial and *Burkholderia* 16S rRNA gene, high amplification efficiency was obtained by qPCR (93–100% and 91–100%, respectively, and r^2 values between 0.995 and 0.999). Relative abundance of *Burkholderia* was calculated as a ratio between *Burkholderia* gene copy numbers by bacterial gene copy numbers (see supporting information for more details).

Amplification and cloning of Burkholderia *16S rRNA gene sequences*

To study the diversity of *Burkholderia* in soil, *Burkholderia* 16S rRNA genes were amplified using the modified primers BKH143F (5′- TGGGGGATAGCYCGGCG –3′) and BKH1434R (5′- TGCGGTTAGRCTAGCYACT –3′) (Schönmann *et al.*, 2009). Cycling conditions were 95°C for 3 min, 40 cycles of 95°C for 60 s, 61.5°C for 60 s, and 72°C for 90 s, final extension at 72°C for 5 min. Reactions were performed in 50 μl volumes containing 1× reaction buffer containing $MgCl_2$ (1.5 mM) (Sigma-Aldrich, St. Louise, MO, USA), 0.8 μM of each primer (Microsynth, Balgach, Switzerland), 0.2 mM dNTP mixture, 0.25 mg ml^{-1} of bovine serum albumin, 2U of Taq DNA Polymerase (Sigma-Aldrich, St. Louise, MO, USA) and 2 μl of template DNA. PCR was carried out in a C1000 Thermal Cycler (Bio-Rad, United Kingdom). PCR products were confirmed by standard 1% agarose gel electrophoresis and gel purified (Gel PCR purification kit, QIAGEN, Hilden, Germany). *Burkholderia* 16S rRNA clone libraries were made from 14 selected locations (Table S2). Purified PCR products were cloned into the pGEM-T Easy vector (Promega, Southampton, United Kingdom). Selected clones from 16S rRNA clone libraries were sequenced using the M13f vector primer.

Sequence analysis

Sequences of chimeric origin were detected by analyzing alignments using Chimera.Slayer and Chimera.UCHIME as implemented by the MOTHUR software (Schloss *et al.*, 2009; Edgar *et al.*, 2011; Haas *et al.*, 2011). Sequences from short or failed reads were excluded from analysis. Sequences were aligned using the SINA web aligner (Pruesse *et al.*, 2007). The alignments were merged into the SILVA SSU reference database release 106 using the ARB software package (Ludwig *et al.*, 2004). Sequences were deposited to the National Center for Biotechnology Information database with accession numbers KC353471 to KC354145. A 50% similarity filter was created for the dataset, based on the alignment, leaving 590 nucleotides for 16S rRNA sequence alignments. The closest cultivated relatives were selected from the reference dataset. Bootstrapped maximum likelihood trees (1000 repetitions) were calculated with sequences affiliated with the groups of interest and close relatives on a dedicated RAXML web server (Stamatakis *et al.*, 2008).

Phylogenetic analysis

Distance matrices were exported to calculate rarefaction curves and diversity indices with the MOTHUR software (Schloss *et al.*, 2009). Sequences were grouped into operational taxonomic units (OTU) using the furthest-neighbour approach, with an OTU defined as containing sequences that are no more than 2% different from each other. This threshold of 98% identity was selected because of the high similarity between *Burkholderia* 16S rRNA sequences over the relatively short read length used in the present study. Richness and diversity were estimated from 16S rRNA gene clone libraries using the Shannon–Weaver diversity index (*H*) (Shannon and Weaver, 1963) and the Simpson diversity index (*D*) (Simpson, 1949). Good's coverage (*C*) was calculated as $C = 1 - (n_1/N)$, where n_1 was the number of clones, which occurred only once in a library of *N* clones (Good, 1953), and relative abundances of major phylogenetic groups were determined.

Statistical analysis

Pearson's product-moment correlations between *Burkholderia* 16S rRNA gene relative abundance and environmental parameters were performed in R 2.12.0 (http://www.r-project.org/). For correlating *Burkholderia* transcontinental distribution, we used the following soil and site characteristics: soil pH, organic C content, C/N ratio, C

mineralization rate, elevation, soil moisture deficit (SMD), mean annual temperature (MAT) and mean annual precipitation (MAP). For correlation analysis at the local scale, pH was the only factor used.

We used Spearman's rank correlation to compare estimate of *Burkholderia* composition with site elevation, soil chemistry (matrix including pH, C/N ratio and percentage of organic C) and climatic (MAT, MAP), soil (percentage of silt and clay, depth of O horizon and SMD), biological (C mineralization rate) and spatial (longitude, latitude) parameters. To estimate the pairwise similarity in *Burkholderia* communities, we generated Bray–Curtis dissimilarity matrices, using rarefied abundance table of *Burkholderia* phylotypes (OTUs) as an input (22 sequences per location). We used the Mantel test in R 2.12.0 to compare dissimilarity matrices to pairwise distances in environmental characteristics as estimated using normalized Euclidean distances in the measured soil and site parameters.

Acknowledgements

The authors would like to thank Prof. Jim Prosser, Dr. Graeme Nicol and Dr. Cécile Gubry-Rangin for valuable discussions, Prof. Jakob Pernthaler, Esther Eckert and Stefan Neuenschwander for help with the ARB software, as well as the Swiss National Science Foundation for funding (grant 31003A-130089 to L.W. and Marie Heim-Vögtlin fellowship to N.B.).

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Detailed experimental procedure.
Fig. S1. Rarefaction curves for *Burkholderia* 16S rRNA gene libraries from 14 different samples. The OTUs were formed at a 98% identity threshold. Dotted vertical line represents the sequence number threshold used for OTU analyses (22 sequences).
Table S1. *In vitro* tests of acid tolerance. 68 *Burkholderia* strains were tested on AB minimal medium supplemented with glucose and agar. Colony formation was selected as growth criterion. Presence or absence of colony formation under various pH conditions is represented in the table with + and – signs respectively.
Table S2. Site description of selected sampling locations from the trans-continental scale study.
Table S3. OTU table with closest relatives. Sequences from each OTU were compared with the database on NCBI using the BLAST tool, and the best hits were selected as closest relatives.

Supporting information

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Detailed experimental procedure

Quantitative PCR

To quantify *Burkholderia* 16S rRNA genes in soil samples, primers BKH812F (5'-CCC TAA ACG ATG TCA ACT AGT TG-3') and BKH1249R (5'-ACC CTC TGT TCC GAC CAT-3') (Bergmark *et al*., 2012) were used. Both primers were tested prior usage for their specificity on DNA isolated from soils with different pH. Annealing temperature of 64 °C was chosen, which gave good amplification and 100% specificity, and was used subsequently in qPCR protocol. Standard curves were generated from known amounts of linearized plasmid (pCR®2.1, Invitrogen) containing *Burkholderia gladioli* 16S rRNA gene fragment amplified by using primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Wilson et al., 1990) in a dilution series of 10^2 to 10^8 copies. Cycling conditions were 95°C for 5 min, 45 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 45 s, followed by collection of fluorescence data and melting curve analysis (64 to 92°C).

Quantification of bacterial 16S rRNA genes was based on the protocol described by Smith *et al*. (2006). Bacterial 16S rRNA genes were amplified with primers 1369F and Prok1492R (Smith *et al*., 2006) and a dilution series (10^2 to 10^9) of linearized plasmid described above was used. Cycling conditions were 95°C for 5 min, 40

cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s, followed by collection of fluorescence data and melting curve analysis (64 to 92°C).
PCR was performed in MicroAmp optical 96-well plates using the automated ABI 7500 Fast sequence detector (Applied Biosystems). Each 25-µl reaction contained the following: 0.25 mg ml-1 bovine serum albumin (BSA), 0.7 µM (Burkholderia 16S rRNA assay) or 0.8 µM (bacterial 16S rRNA assay) primers, 12.5 µl of SYBR® Green PCR master mix (ABI, Warrington, United Kingdom), 5 µl of nucleic acid template and additionally 2 mM MgCl2 for Burkholderia 16S rRNA assay.

Each plate included triplicate reactions per DNA sample and the appropriate set of standards. After the DNA amplification cycles, melting curve analysis was performed to confirm that the obtained signals were caused by the specific amplicon and additionally PCR products were confirmed by standard 1% agarose gel electrophoresis The CT values for each PCR reaction were automatically calculated and analysed by the ABI prism sequence detection systems software (version 2.0). A standard curve was obtained by plotting CT values as a function of log-transformed copy numbers of linearized plasmid. There was a linear relationship between the log of the plasmid DNA copy number and the Ct values across the specified concentration range (r^2 values between 0.995 and 0.999) and a slope of 3.55 to 3.32 and 3.50 to 3.32 (data not shown) for *Burkholderia* 16S rRNA assay and bacteria 16S rRNA assay, indicating a high amplification efficiency between 91 to 100% and 93 to 100%, respectively. Data were presented as ratio between *Burkholderia* copy numbers and bacteria copy numbers.

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Table S1.

			pH of AB media supplemented with glucose									
Species	Strain ID	Origin of isolation	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**	**8.0**
Burkholderia ambifaria	LMG19182	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia ambifaria	LMG19467	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia andropogonis	LMG2129	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia anthina	LMG20980	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia anthina	LMG20983	clinical	-	+	+	+	+	+	+	+	+	+
Burkholderia caledonica	LMG19076	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia caribensis	LMG18531	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia caryophylli		environment	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG16656	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	ZYB002	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG16659	clinical	-	-	+	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG18829	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG18830	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG16654	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG18832	clinical	-	+	+	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG18828	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG16656	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia		clinical	-	+	+	+	+	+	+	+	+	+
Burkholderia cenocepacia	LMG18863	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia cenocepacia	H111	clinical	-	-	+	+	+	+	+	+	+	+
Burkholderia cepacia	LMG1222	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia cepacia	IST408	clinical	-	-	+	+	+	+	+	+	+	+
Burkholderia cepacia	LMG1222	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia cepacia	1S18	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia dolosa	LMG21443	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia dolosa	LMG21820	clinical	-	+	+	+	+	+	+	+	+	+
Burkholderia fungorum		environment	-	-	-	+	+	+	+	+	+	+
Burkholderia fungorum	LMG16225	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia gladioli	LMG2216	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia gladioli	LMG2216	clinical	-	+	+	+	+	+	+	+	+	+
Burkholderia gladioli	LMG11626	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia gladioli	1S8	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia glathei	LMG14190	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia glumae	LMG2196	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia glumae	ATCC33617	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia glumae	AU6208	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia graminis		environment	-	-	-	+	+	+	+	+	+	+
Burkholderia graminis		environment	-	-	-	+	+	+	+	+	+	+
Burkholderia graminis	LMG18924	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia hospita	LMG20598	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia kururiensis	LMG19447	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia lata	LMG6993	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia lata	ATCC17660	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia multivorans	LMG18825	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia phenazinium	S1	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia phenazinium	A10	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia phenazinium	LMG2247	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia phytofirmans	LMG22487	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia plantarii	ATCC43733	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia plantarii	LMG9035	environment	-	+	+	+	+	+	+	+	+	+
Burkholderia pyrrocinia	LMG21822	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia pyrrocinia	LMG14191	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia pyrrocinia	LMG21822	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia sacchari	LMG19450	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia sordidicola	LMG22029	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia stabilis	LMG7000	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia stabilis	LMG14294	clinical	-	-	-	+	+	+	+	+	+	+
Burkholderia terricola		environment	-	-	-	+	+	+	+	+	+	+
Burkholderia terricola	ZR2-12	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia terricola	LMG20594	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia terricola	A25	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia thailandensis	LMG20219	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia tropica	LMG22274	environment	-	-	+	+	+	+	+	+	+	+
Burkholderia ubonensis	LMG20358	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia ubonensis	LMG24263	clinical	-	-	+	+	+	+	+	+	+	+
Burkholderia vietnamiensis	LMG18835	clinical	-	-	+	+	+	+	+	+	+	+
Burkholderia vietnamiensis	LMG16232	environment	-	-	-	+	+	+	+	+	+	+
Burkholderia xenovorans	LMG21463	environment	-	-	-	+	+	+	+	+	+	+

Table S2.

Soil ID	Location Description	site latitude (deg)	site latitude (min)	site longitude (deg)	site longitude (min)	site elevation	dominant plant species	vegetation type	soil order	depth of O horizon	MAT	MAP	SMD	Ecosystem Domain descriptor	Ecosystem division descriptor	Ecosystem Domain	% organic C	C:N ratio	% silt + clay	texture class	pH	C min rate
AR1	Misiones, Argentina	27	44	55	41	0	Leguminosae, Eugenia sp.	deciduous/broadleaf forest	oxisol	4	23	1400	-206	Humid Tropic	Rainforest - Mountains	Humid Tropical	2.21	8.98	80	clay	5.8	4.3
BB1	Bear Brook, ME, USA	44	52	68	6	400	Picea rubens	coniferous forest	spodosol	16	6.1	1200	-680.2	Humid Tempe	Warm Continental - Mountains	Humid Temperate	12.84	20.5	41	sandy loam	4.25	5.610
BF1	Bousson Forest, PA, USA	41	35	80	3	390	Acer saccharum, Fagus grandifolia, Prunus serotina	deciduous/broadleaf forest	alfisol	11.6	7.8	1000	-451.3	Humid Tempe	Warm Continental	Humid Temperate	6.44	13.7	60	loam	4.05	7.019
BF2	Bousson Forest, PA, USA	41	35	80	3	390	Acer saccharum, Fagus grandifolia, Prunus serotina	deciduous/broadleaf forest	alfisol	6.1	7.8	1000	-451.3	Humid Tempe	Warm Continental	Humid Temperate	9.52	15.2	56	loam	3.61	8.718
BZ1	Bonanza Creek LTER, AK USA	64	48	148	15	300	Picea glauca	coniferous forest	gelisol	7.5	-2.9	260	132.6	Polar Forest	Subarctic	Polar	3.03	19.4	80	silt loam	5.12	4.185
BZ2	Bonanza Creek LTER, AK USA	64	48	148	15	300	Picea glauca	coniferous forest	gelisol	7.5	-2.9	260	132.6	Polar Forest	Subarctic	Polar	3.03	20.5	79	silt loam	5.16	8.064
BZ3	Bonanza Creek LTER, AK USA	64	48	148	15	300	Picea glauca	coniferous forest	gelisol	7.5	-2.9	260	132.6	Polar Forest	Subarctic	Polar	3.73	20.8	49	silt loam	5.36	10.020
CC1	Cedar Creek LTER, MN USA	45	24	93	12	110	Carex muhlenbergii, Andropogon gerardii, Poa pratensis,Schizachyrium scoparium	grassland	inceptisol	0	5.8	720	-143.3	Humid Tempe	Warm Continental	Humid Temperate	1.91	14.5	11	sand	6.06	4.204
CF1	Catskills, NY USA	42	9.5	74	15.5	800	Acer saccharum, Fagus grandifolia, Acer rubrum	deciduous/broadleaf forest	inceptisol	6	5.3	1300	-858.7	Humid Tempe	Warm Continental - Mountains	Humid Temperate	2.56	13.6	49	loam	3.92	2.266
CF3	Catskills, NY USA	42	7	74	6	800	Tsuga canadensis	coniferous forest	inceptisol	9	5.3	1300	-858.7	Humid Tempe	Warm Continental - Mountains	Humid Temperate	4.33	17.0	77	silt loam	3.56	2.912
CL1	Calhoun Experimental Forest, SC USA	34	37	81	40	150	Quercus spp., Carya spp., Acer rubrum	deciduous/broadleaf forest	ultisol	3	15.9	1250	-420.1	Humid Tempe	Subtropical	Humid Temperate	2.33	19.6	35	sandy loam	5.68	4.642
CL4	Calhoun Experimental Forest, SC USA	34	37	81	40	150	Hordeum sp.	grassland	ultisol	0	15.9	1250	-420.1	Humid Tempe	Subtropical	Humid Temperate	1.71	13.7	36	sandy loam	5.03	2.234
CO3	Shortgrass Steppe LTER, CO USA	40	48	104	50	1500	Bouteloua gracilis, Buchloe dactyloides, Sphaeralcea coccinea	grassland	mollisol	0	9.3	322	230.6	Dry Grasslan	Temperate Steppe	Dry	0.82	11.7	24	sandy loam	6.02	2.443
DF1	Duke Forest, NC USA	35	58	79	5	163	Pinus taeda, Liquidambar styraciflua, Liriodendron tulipifera, Cornus florida (Pinus overstory, hardwood understory)	coniferous forest	alfisol	4	14.6	1100	-328.0	Humid Tempe	Subtropical	Humid Temperate	2.78	37.7	43	sandy loam	5.37	13.201
DF3	Duke Forest, NC USA	35	58	79	5	150	Quercus alba	deciduous/broadleaf forest	alfisol	2	14.6	1100	-328.0	Humid Tempe	Subtropical	Humid Temperate	1.70	25.8	20	loamy sand	5.05	4.149
GB2	Great Basin Experimental Range, UT USA	39	19	111	28	3290	Populus tremuloides, Vicia americana, Bromus inermis understory	deciduous/broadleaf forest	mollisol	2	2.0	400	-112.3	Dry Forest	Temperate Steppe - Mountains	Dry	6.89	13.1	65	loam	7.57	5.375
GB3	Great Basin Experimental Range, UT USA	39	19	111	29	3270	Abies concolor, Picea engelmannii	coniferous forest	mollisol	4	2.0	400	-112.3	Dry Forest	Temperate Steppe - Mountains	Dry	5.71	14.3	76	clay loam	7.18	6.244
HI2	Kohala Peninsula, HI USA	20	5	155	42	700	Pennisetum clandestinum	grassland	andisol	0	22.8	750	357.6	Humid Tropic	Rainforest - Mountains	Humid Tropical	15.88	11.0	36	sandy loam	6.32	11.805
HI3	Kohala Peninsula, HI USA	20	5	155	42	1000	Pennisetum clandestinum	grassland	andisol	0	22.8	1000	107.6	Humid Tropic	Rainforest - Mountains	Humid Tropical	18.24	11.2	25	loamy sand	6.53	13.285
HJ1	H.J. Andrews Experimental Forest, OR USA	44	13	122	9	700	Pseudotsuga menziesii, Tsuga heterophylla, Thuja plicata	coniferous forest	andisol	1	9.4	2000	-1507.0	Humid Tempe	Marine - Mountains	Humid Temperate	6.95	36.6	41	sandy loam	5.41	5.079
HJ2	H.J. Andrews Experimental Forest, OR USA	44	13	122	9	700	Alnus rubra, Rhododendron macrophyllum	deciduous/broadleaf forest	andisol	1	9.4	2000	-1507.0	Humid Tempe	Marine - Mountains	Humid Temperate	7.61	26.1	47	sandy loam	5.36	4.532
IE1	Institute for Ecosystem Studies, NY USA	41	48	73	45	75	Poa pratense, Galium aparine, Solidago sp.	grassland	inceptisol	0.5	8.6	1200	-609.9	Humid Tempe	Warm Continental	Humid Temperate	2.70	11.8	49	sandy loam	5.27	7.536
IE2	Institute for Ecosystem Studies, NY USA	41	48	73	45	75	Antoxanthum odoratum, Cornilla varia, Galium aparine, Schizachyrum scoparium	grassland	inceptisol	0.1	8.6	1200	-609.9	Humid Tempe	Warm Continental	Humid Temperate	4.07	13.2	49	sandy loam	5.52	11.422
IT1	Itasca Lake State Park, MN USA	47	10	95	10	550	Acer saccharum, Corylus cornuta, Populus tremuloides	deciduous/broadleaf forest	spodosol	1	3.0	750	-262.1	Humid Tempe	Warm Continental	Humid Temperate	6.31	22.5	27	sandy loam	5.78	16.079
IT2	Itasca Lake State Park, MN USA	47	11	95	10	550	Pinus resinosa	coniferous forest	spodosol	2	3.0	750	-262.1	Humid Tempe	Warm Continental	Humid Temperate	3.91	23.0	19	loamy sand	5.42	16.003
JT1	Joshua Tree National Park, CA USA	33	58	116	4	1360	Larrea tridentata, Ambrosia dumosa	shrubland	aridisol	0	16	90	1032	Dry Shrublan	Tropical/Subtropical Desert	Dry	.687	8	18	loamy sand	7.6	11.7
KP1	Konza Prairie LTER, KS USA	39	6	96	36	100	Andropogon gerardii, Sorghastrum nutans, Poa pratensis	grassland	mollisol	1.5	12.5	835	-80.5	Humid Tempe	Prairie	Humid Temperate	6.12	13.6	78	silt loam	6.37	4.324
KP2	Konza Prairie LTER, KS USA	39	6	96	36	100	Andropogon gerardii, Sorghastrum nutans, Andropogon scoparius	grassland	mollisol	0	12.5	835	-80.5	Humid Tempe	Prairie	Humid Temperate	4.62	13.4	77	silt loam	6.5	3.603
LQ1	Luquillo LTER, Puerto Rico	18	18	65	50	1000	Tabebuia ridgida	deciduous/broadleaf forest	inceptisol	6	19.3	5000	-4111.8	Humid Tropic	Rainforest - Mountains	Humid Tropical	13.95	24.6	64	silt loam	4.89	5.950
MD2	Mojave Desert, CA USA	34	54	115	36	1171	arrea tridentata, Ambrosia dumosa, Yucca shidigera, Bromus spp.	shrubland	aridisol	0	21.0	150	1053.8	Dry Shrublan	Tropical/Subtropical Desert	Dry	0.42	8.8	26	sandy loam	7.65	1.970
MD5	Mojave Desert, CA USA	35	12	115	52	776	Larrea tridentata, Ambrosia dumosa, Yucca shidigera, Bromus spp.	shrubland	aridisol	0	21.0	150	1086.7	Dry Shrublan	Tropical/Subtropical Desert	Dry	0.57	9.5	20	loamy sand	8.07	5.931
PE2	Manu National Park, Peru	13	5	71	35	2750	Cyatheaceae, Cunoniaceae, Euphorbiaceae	deciduous/broadleaf forest	inceptisol	50	12	2500	-2940	Tropical Fore	Rainforest - Mountains	Humid Tropical	5.74	15.36	34	sandy loam	4.11	14.4
PE3	Manu National Park, Peru	13	5	71	35	2000	Cyatheaceae, Clusiaceae, Lauraceae	deciduous/broadleaf forest	inceptisol	50	16	5500	-3270	Tropical Fore	Rainforest - Mountains	Humid Tropical	13.09	16.84	40	sandy loam	4.25	31.8
PE5	Manu National Park, Peru	12	38	71	16	860	Maieta sp, Ficus sp.	deciduous/broadleaf forest	oxisol	0	23	5000	-1900	Tropical Fore	Rainforest - Mountains	Humid Tropical	9.36	14.55	70	clay loam	3.57	25.0
PE7	Manu National Park, Peru	12	39	71	14	366	Cecropia sp., Begonia sp., Brosimum sp.	deciduous/broadleaf forest	oxisol	0	25	4000	-1600	Tropical Fore	Rainforest - Mountains	Humid Tropical	6.377	11.21	88	silty clay	5.51	16.7
SK1	BOREAS site, Saskatchewan, Canada	53	54	104	42	579	Pinus banksiana	coniferous forest		5	0.4	467	-13	Polar Forest			1.16	67	21	loamy sand	5.45	5.8
SK2	BOREAS site, Saskatchewan, Canada	53	59	105	12	628	Picea mariana	coniferous forest		30	0.4	467	-13	Polar Forest			2.49	5	35	sandy loam	6.18	6.5
SK3	BOREAS site, Saskatchewan, Canada	53	36	106	12	601	Populus tremuloides	deciduous/broadleaf forest		10	0.4	467	-13	Polar Forest			.85	5	55	loam	5.83	3.7
SR1	Sedgwick Reserve, CA USA	34	42	120	3	300	Quercus douglasii with Brome sp. understory	deciduous/broadleaf forest	mollisol	2	17.2	500	323.8	Dry Shrublan	Mediterranean	Humid Temperate	4.59	11.1	55	loam	6.84	6.119
SR2	Sedgwick Reserve, CA USA	34	41	120	2	300	Artemisia california and Salvia leucophylla	shrubland	mollisol	0.1	17.2	500	323.8	Dry Shrublan	Mediterranean	Dry	1.46	11.1	48	sandy loam	8.00	2.461
SR3	Sedgwick Reserve, CA USA	34	41	120	3	300	Bromus spp., Hordeum murinum	grassland	mollisol	1	17.2	500	323.8	Dry Shrublan	Mediterranean	Humid Temperate	3.30	11.0	61	loam	6.95	7.154
TL1	Toolik Lake LTER, AK USA	68	38	149	35	894	Eriophorum vaginatum	grassland	gelisol	0	-9.3	400	-211.8	Polar Grassla	Tundra	Polar	7.02	18.7	57	loam	4.58	9.241
TL2	Toolik Lake LTER, AK USA	68	38	149	35	894	Salix spp., Betula nana	shrubland	gelisol	0	-9.3	400	-211.8	Polar Shrubla	Tundra	Polar	15.83	16.6	61	silt loam	6.47	12.092
TL3	Toolik Lake LTER, AK USA	68	38	149	35	894	Vaccinium spp.	shrubland	gelisol	0	-9.3	400	-211.8	Polar Shrubla	Tundra	Polar	5.39	24.6	52	loam	4.23	16.456

OTUs	BF1	BF2	CL1	CL4	DF1	HI3	HJ1	KP2	PE5	SK1	SK2	SK3	SR1	TL3	total # sequences	NCBI accession number	Identity of the closest relative sequence
OTU001	8	6	1	0	0	0	51	0	0	53	19	23	1	28	190	AY154369	*Burkholderia glathei* ; Hg 4
OTU002	0	0	0	0	0	0	0	53	0	0	0	0	0	0	53	AY040362	*Burkholderia terricola* (T); LMG 20594T
OTU003	0	0	0	0	0	33	0	0	0	0	0	0	0	0	33	U96935	*Burkholderia glathei* (T); LMG14190T
OTU004	0	1	1	0	3	0	12	0	0	0	0	4	1	10	32	AY154370	*Burkholderia glathei* ; Hg 5
OTU005	5	7	8	1	1	0	4	0	0	2	0	1	0	0	29	AY154372	*Burkholderia phenazinium* ; Hg 8
OTU006	7	12	0	1	0	0	0	0	0	1	5	0	0	1	27	AJ544691	*Burkholderia fungorum* ; W566
OTU007	1	0	10	1	7	0	0	0	2	0	0	0	4	1	26	AB201284	*Burkholderia terrae* ; KMY01
OTU008	5	3	0	1	5	0	0	0	0	0	3	0	0	0	17	AY040353	*Burkholderia hospita* ; LMG 20574
OTU009	0	0	0	12	0	0	0	0	3	0	0	0	0	0	15		*Burkholderia cepacia* complex
OTU010	8	3	3	0	0	0	0	0	0	0	0	0	0	0	14	AF061872	*Burkholderia sartisoli* (T); RP007
OTU011	1	5	5	0	2	0	0	0	0	0	0	0	0	0	13	AM502993	*Burkholderia phenazinium* ; WT1 11
OTU012	3	7	2	0	0	0	0	0	0	0	0	0	0	0	12	U96936	*Burkholderia phenazinium* (T); LMG2247T
OTU013	0	0	4	0	3	0	0	0	0	0	1	4	0	0	12	AJ292639	uncultured eubacterium WD227
OTU014	0	7	0	0	1	0	0	0	0	0	0	0	0	0	8	DQ419958	*Burkholderia* sp. DM-Ni1
OTU015	0	0	0	8	0	0	0	0	0	0	0	0	0	0	8	DQ465451	*Burkholderia soli* (T); GP25-8
OTU016	1	0	4	1	1	0	0	0	0	0	0	0	0	0	7	Y17010	*Burkholderia caribensis* ; MWAP71
OTU017	0	0	0	1	2	0	0	0	0	0	0	4	0	0	7	AF508806	*Burkholderia* sp. TNFYE-5
OTU018	0	0	0	0	0	0	0	0	0	0	0	0	7	0	7	AY497470	*Burkholderia phytofirmans* (T); PsIN
OTU019	0	0	0	1	0	0	0	0	5	0	0	0	0	0	6	AY773196	*Burkholderia mimosarum* ; Br3467
OTU020	0	0	0	0	0	0	0	0	6	0	0	0	0	0	6	AY221956	*Burkholderia unamae* (T); MTI-641
OTU021	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4	AB201284	*Burkholderia terrae* ; KMY01
OTU022	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4	AF164045	*Burkholderia tropica* ; AB98
OTU023	0	0	0	0	0	0	0	0	0	0	0	4	0	0	4	EF018321	uncultured *Burkholderiaceae* bacterium
OTU024	1	1	1	0	0	0	0	0	0	0	0	0	0	0	3	CP001043	*Burkholderia phymatum* STM815
OTU025	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	FJ466275	uncultured bacterium; E42
OTU026	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3	AY307366	*Burkholderia* sp. M14
OTU027	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	DQ490298	*Burkholderiaceae* bacterium KVD-unk-48
OTU028	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	AY904767	*Burkholderia* sp. SEMIA 6166
OTU029	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	FJ772007	*Burkholderia* sp. IMER-B2-9
OTU030	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	AF408962	*Burkholderia* sp. Ellin120
OTU031	0	0	0	0	0	0	1	0	0	0	0	0	0	2	3	DQ378169	uncultured beta proteobacterium; F03_Pitesti
OTU032	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	HQ728571	*Burkholderia* sp. Os40
OTU033	0	0	0	0	0	0	1	0	0	0	2	0	0	0	3	U96936	*Burkholderia phenazinium* (T); LMG2247T
OTU034	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3	HM640291	*Burkholderia glathei* ; YUST-DW12
OTU035	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	AY326593	uncultured soil bacterium; 142-1
OTU036	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3	HQ728571	*Burkholderia* sp. Os40
OTU037	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	X67039	*Burkholderia caryophylli* ; ATCC 25418
OTU038	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2	AB578860	*Burkholderia* sp. H6-1
OTU039	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	HM110857	uncultured beta proteobacterium; SHNW635
OTU040	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	DQ490297	*Burkholderiaceae* bacterium KVD-unk-47
OTU041	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	JN590319	*Burkholderia* sp. Juv935
OTU042	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	GU292560	*Burkholderia* sp. FA2
OTU043	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	FJ772007	*Burkholderia* sp. IMER-B2-9
OTU044	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	AB531407	*Burkholderia* sp. II-44
OTU045	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	FN543658	*Burkholderia* sp. JPY168
OTU046	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	AY773194	*Burkholderia* sp. Br3464
OTU047	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	EU024154	*Burkholderia graminis* ; CIP 106649
OTU048	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	DQ256491	*Burkholderia sordidicola* ; Jm120
OTU049	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	AY154370	*Burkholderia glathei* ; Hg 5
OTU050	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	DQ256491	*Burkholderia sordidicola* ; Jm120
OTU051	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	HM640291	*Burkholderia glathei* ; YUST-DW12
OTU052	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	AY691394	*Burkholderia* sp. hpud10.4
OTU053	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	AJ300687	*Burkholderia* sp. K14
OTU054	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	AF052387	*Burkholderia* sp. 'SAP II'
OTU055	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	GQ306172	*Burkholderia* sp. enrichment culture clone
OTU056	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	AB212228	*Burkholderia* sp. C308
OTU057	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	AB232330	*Burkholderia* sp. KFA1
OTU058	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	DQ451490	uncultured bacterium; FAC51
OTU059	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	AY154378	*Burkholderia glathei* ; Hg 18
OTU060	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	FJ528270	*Burkholderia* sp. Gc145
OTU061	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AB025790	*Burkholderia* sp. NF100
OTU062	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	DQ465451	*Burkholderia soli* (T); GP25-8
OTU063	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AJ300689	*Burkholderia* sp. 135M
OTU064	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AY769902	*Burkholderia* sp. KBC-1
OTU065	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	X67039	*Burkholderia caryophylli* ; ATCC 25418
OTU066	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	FJ603036	*Burkholderia* sp. Ny10
OTU067	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AJ292639	uncultured eubacterium WD227
OTU068	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	FJ772007	*Burkholderia* sp. IMER-B2-9
OTU069	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AF408965	*Burkholderia* sp. Ellin123
OTU070	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	CP000151	*Burkholderia* sp. 383
OTU071	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	DQ490297	*Burkholderiaceae* bacterium KVD-unk-47
OTU072	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	DQ490297	*Burkholderiaceae* bacterium KVD-unk-47
OTU073	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AF097533	*Burkholderia stabilis* (T); LMG 14294
OTU074	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	AY752953	*Burkholderia mimosarum* ; PTU10
OTU075	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	DQ472169	*Burkholderia* sp. SH-1
OTU076	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	FJ603036	*Burkholderia* sp. Ny10
OTU077	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	AY178076	*Burkholderia* sp. CD9
OTU078	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	EU219865	*Burkholderia* sp. WSM3937
OTU079	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	AY533863	*Burkholderia* sp. ; BR3469
OTU080	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	DQ419958	*Burkholderia* sp. DM-Ni1
OTU081	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	AY040353	*Burkholderia hospita* ; LMG 20574
OTU082	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	DQ419959	*Burkholderia* sp. DM-Ni2
OTU083	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	DQ419958	*Burkholderia* sp. DM-Ni1
OTU084	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	X67039	*Burkholderia caryophylli* ; ATCC 25418
OTU085	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	AB201284	*Burkholderia terrae* ; KMY01
OTU086	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	AJ292647	uncultured eubacterium WD2114
OTU087	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AF448045	*Burkholderia* sp. SOD-6
OTU088	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY691394	*Burkholderia* sp. hpud10.4
OTU089	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AJ971352	*Burkholderia* sp. C1
OTU090	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY773185	*Burkholderia sabiae* ; Br3405
OTU091	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	DQ514537	*Burkholderia ferrariae* (T); FeGl01
OTU092	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY773194	*Burkholderia* sp. Br3464
OTU093	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY773189	*Burkholderia nodosa* (T); Br3437
OTU094	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY773191	*Burkholderia mimosarum* ; Br3454
OTU095	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY691397	*Burkholderia* sp. hpig15.6
OTU096	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AJ302312	*Burkholderia phymatum* (T); STM815
OTU097	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AF164045	*Burkholderia tropica* ; AB98
OTU098	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AY326594	uncultured soil bacterium; 1048-2
OTU099	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AB021423	*Burkholderia caryophylli* (T); ATCC 25418T
OTU100	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	U96941	*Burkholderia graminis* ; AUS35
OTU101	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	AB030584	*Burkholderia ubonensis* (T); GTC-P3-415
OTU102	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	AY154373	*Burkholderia phenazinium* ; Hg 10
OTU103	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	AJ292637	uncultured eubacterium WD202
OTU104	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	AJ292637	uncultured eubacterium WD202
OTU105	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	AY154370	*Burkholderia glathei* ; Hg 5
OTU106	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	AF448045	*Burkholderia* sp. SOD-6
OTU107	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	AJ292647	uncultured eubacterium WD2114
OTU108	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	AF184926	2,4-D-degrading bacterium TFD13
OTU109	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	AY178076	*Burkholderia* sp. CD9
OTU110	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AJ292639	uncultured eubacterium WD227
OTU111	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY154373	*Burkholderia phenazinium* ; Hg 10
OTU112	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY178076	*Burkholderia* sp. CD9
OTU113	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY178076	*Burkholderia* sp. CD9
OTU114	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AJ292647	uncultured eubacterium WD2114
OTU115	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY238505	*Burkholderia* sp. 13
OTU116	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AJ292646	uncultured eubacterium WD2109
OTU117	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY178062	*Burkholderia* sp. UCT 29
OTU118	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	U96935	*Burkholderia glathei* (T); LMG14190T
OTU119	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY154373	*Burkholderia phenazinium* ; Hg 10
OTU120	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY178076	*Burkholderia* sp. CD9
OTU121	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	AY238505	*Burkholderia* sp. 13
OTU122	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	AJ292647	uncultured eubacterium WD2114
OTU123	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Y17052	*Burkholderia glathei* ; ATCC 29195T; LMG 14190T

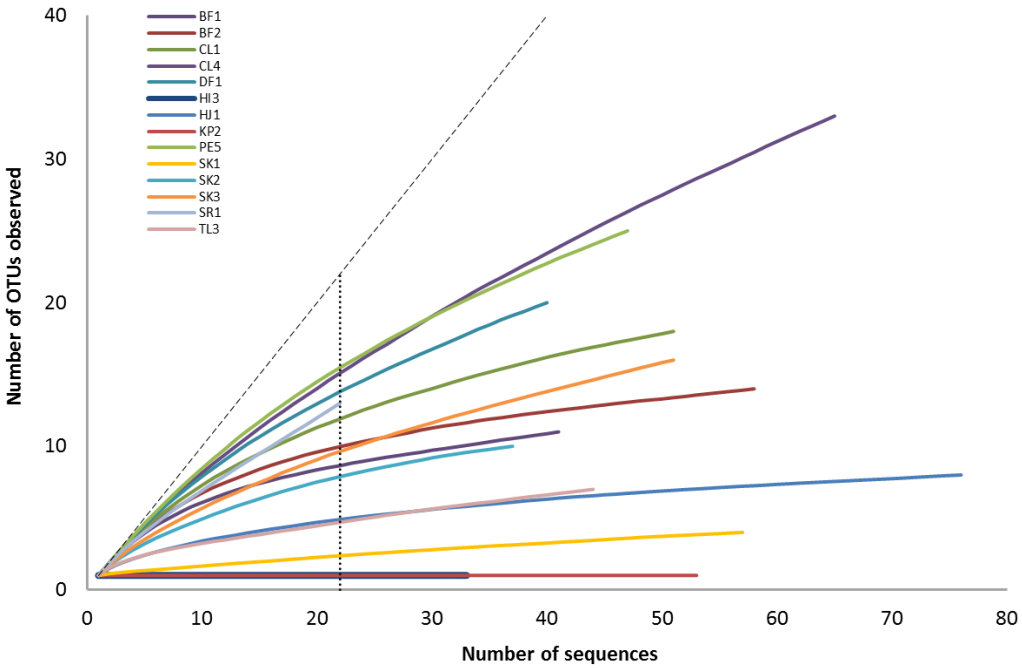

Figure S1: Rarefaction curves for *Burkholderia* 16S rRNA gene libraries from 14 different samples. The OTUs were formed at a 98% identity threshold. The dotted vertical line represents the sequence number threshold used for OTU analyses (22 sequences).

Part II: *Burkholderia*-fungi interactions

Aims of the project

Studies have shown that *Burkholderia* are able to interact with fungi either in an antagonistic or in a symbiotic/mutualistic way. The latter interactions are of particular interest in the soil environment, as they can provide a number of benefits to both partners in an otherwise very competitive and nutrient limited system. Recent publications investigating the potential ecological role of such interactions have shown that *Burkholderia* benefit from the interaction with fungi in various ways. Fungal exudates have two important roles in these interactions; i) they can be utilized by *Burkholderia* and ii) they can induce a local pH increase. Additionally, *Burkholderia* are able to use fungal hyphae for translocation and dispersal. However most of this knowledge came from studies investigating specific interaction between *B. terrae* and *Lyophyllum* sp. Karst. Thus it is not known if other *Burkholderia* species are also able to interact with fungi, to profit from this interaction and how this lifestyle changes their physiology. To answer these questions, we used a combination of bioinformatics, cultivation-based methods and proteomics to understand the nature of these interactions. Co-occurrence network analyses provided the answer as to how frequent these interactions might be in soils and allowed to select candidate model species for further work. Growth experiments with co-cultures using different fungi and *Burkholderia* species enabled to assess whether the capacity to colonize hyphae and migrate with them is specific to certain *Burkholderia* and fungal species and which lifestyle (growth on the hyphae vs. growth on the medium) is preferred by *Burkholderia*. Finally a global study enabled us to investigate the changes in the proteome of *Burkholderia* when growing with fungi compared to growth alone, and link those changes to ecological reasons why such interactions between *Burkholderia* and fungi might be important in acidic soils, a habitat shared by both types of organisms.

Unpublished results

Effect of fungal presence and absence on Burkholderia *relative abundance in the soil*

To investigate the relationship between *Burkholderia* and fungi and to test the hypothesis that beneficial interactions are formed between these two organisms we designed microcosm experiments. In sterile soils, the survival of *Burkholderia* species has been shown to depend on fungal presence/growth in the case of *B. terrae* and *Lyophyllum* sp. Karst (Nazir *et al*. 2010). However it is not yet known if such strong dependence would be detected also in native soils, harboring large bacterial communities with hypothetically also a large number of benefiting partners. Unknown soil biodiversity and links between soil microbiota could represent problems in interpretation of the obtained results, however this might be negligible, if similar trends could be observed across large number of samples obtained from different ecosystems. An additional challenge is to find the best way to manipulate the soils to selectively inhibit specific communities. We decided to us antifungal compounds, nystatin and cycloheximide that have been previously applied in similar experiments and have been shown to have broad target specificity towards fungi (Badalucco *et al*. 1994, Adetutu *et al*. 2012). With these two compounds we were able to reduce fungal biomass without directly affecting *Burkholderia* and the rest of the bacterial community.

Methods and material

Construction of microcosms containing antifungal compounds

In the microcosm experiment described above (page 20), we included an additional series of microcosms where nystatin solution (Sigma Aldrich, USA) in concentration of 150 and 300 μl g^{-1} dry $soil^{-1}$ was added to the pH-adjusted soils, to inhibit fungal growth. After the incubation, dilutions of the soil were plated on malt extract agar (MEA) and potato dextrose agar (PDA) to verify if the used concentrations of nystatin were high enough to inhibit fungal growth. The complete inhibition was achieved only by using 300 μl g^{-1} dry $soil^{-1}$ of nystatin. The treated soil with 300 μl g^{-1} dry $soil^{-1}$ of nystatin was incubated for 28 days as described above.

For the final experiment, soils collected from San Vittore and Gränichen (both in Switzerland) were selected, with pH 5.45 and 5.9, respectively. Prior to constructing the soil microcosms, we needed to determine the combination and concentration of antifungal compounds needed to completely inhibit fungal growth as these two soils differed significantly from the soils used in the previous experiment. To this end, a set of soils was treated with cycloheximide, nystatine or a combination of both in concentrations of 2 mg g^{-1} soil and 12 μg g^{-1} soil, respectively. These soils were then incubated for up to 10 days in the dark and at the room temperature. After incubation, soil serial dilutions were plated on two fungal culture media (MEA and PDA) to determine which combination of antifungals inhibited totally the fungal growth. This was achieved only by using the combination of both. The microcosms were constructed in 5 replicates using 10 g of soil that was treated with nystatine (2 mg g^{-1} soil) and cycloheximide (12 μg g^{-1} soil) or treated with equal amount of sterilized distilled water (control). Microcosms were incubated for 21 days. Water loss, monitored as change in the weight, was calibrated with addition of sterile distilled water in 3-days intervals.

Quantification of *Burkholderia* relative abundance

To isolate DNA from the soil microcosms, we used a protocol described by Griffiths *et al*. (2000) with some modifications (Nicol *et al*. 2005). DNA was retrieved from the control and the treated microcosms after one day and after 21 or 28 days of incubation. Abundance of *Burkholderia* and bacteria was quantified as described above. *Burkholderia* relative abundance was calculated and presented as ratio between *Burkholderia* and bacteria gene copy numbers g^{-1} dry soil.

Results and discussion

To test our hypotheses a complete inhibition of fungal growth was needed, which was achieved in the first experiment only in the microcosms where nystatin was applied in higher concentrations. Interestingly, the relative abundance of *Burkholderia* decreased significantly upon addition of nystatin, regardless of pH change (Figure 4A). However, since we only applied nystatin to soils where pH had been artificially altered, which had caused shifts in *Burkholderia* and in other members of the bacteria

communities, we could not clearly demonstrate that the observed decrease in *Burkholderia* abundance was only due to beneficial effects of fungi. Therefore, we conducted another microcosm experiment where only antifungals were added to the sampled soil, without any concomitant pH manipulation.

To this end, we used two soils that were sampled on two different locations in Switzerland. In these soils, only dual application of two antifungal agents, nystatin and cycloheximide allowed complete inhibition of fungal growth. However, in presence of both antifungal compounds, *Burkholderia* relative abundance was not decreased as observed previously, but on the contrary, it was significantly increased (Figure 4B). This might suggest an inhibiting effect of fungi on *Burkholderia*. However, a more likely explanation for this observation could lie in the storage condition of the soil samples before the experiment. Soils were collected, sieved and homogenized 6 months prior to the construction of the microcosms and were kept for this whole duration at 4°C. Prolonged incubation at low temperature is known to affect microbial communities. In addition to storage temperature, low nutrient availability is likely to have an even bigger impact on the soil microflora (Krashevska *et al*. 2014, Paula *et al*. 2014). With no inflow of nutrients to the system, as all roots and visible (organic) particles were removed, the bacterial and fungal community structures must have changed significantly, which could bias the results obtained in the second microcosm experiment.

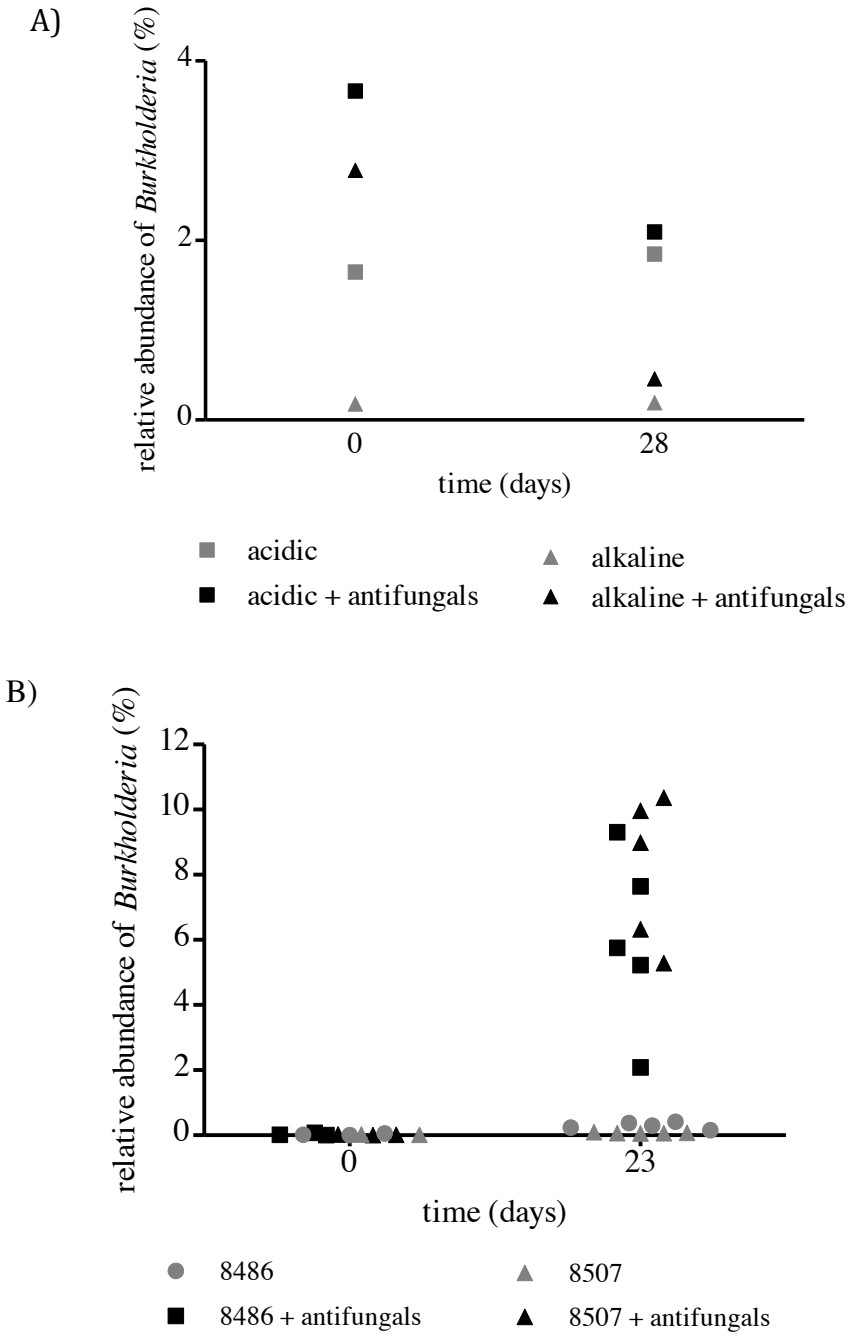

Figure 3. Relative abundance of *Burkholderia* in microcosms treated with antifungal compounds. Relative abundance of *Burkholderia* in microcosms treated with antifungal compounds. A) Soils collected from Craibstone estate were treated with nystatin and incubated for 28 days. Additionally, the pH of the acidic and alkaline soil was adjusted using $Ca(OH)_2$ or H_2SO_4. B) Soils from sampling locations at San Vittore (8486) and Gränichen (8507) were treated with antifungal compounds (nystatin + cycloheximide) and incubated for 23 days.

Genome of B. glathei *LMG14190*

To identify MS/MS spectra of the proteins extracted in our dual culture assay (see below, Published results) a database needed to be created, which contained the protein sequences from the strains used or at least from phylogenetically closely related strains. Some genomes of *Burkholderia* sp., especially those of strains pathogenic to animals and plants, were available. However, as phylogenetic analysis of the genus *Burkholderia* showed that *B. glathei* is not closely related to other *Burkholderia* species, we sequenced the genome of *B. glathei* LMG14190 to avoid misidentification and misinterpretation of the obtained MS/MS protein spectra.

Materials and methods

Detailed information regarding genome sequencing, assembly and annotation is described below (Published results). In short, genetic material was extracted from pure cultures of *B. glathei* LMG14190, purified and processed with RNase to avoid RNA contamination. High quality DNA was further processed at the Functional Genomics Center Zurich (FGCZ) using the Nextera XT kit (Illumina, San Diego, CA, USA). Obtained sequencing reads were assembled using the SPADES v2.5 software (Nurk *et al*., 2013). The resulting 138 contigs were further processed to the annotation step using the Prodigal software (Hyatt *et al*., 2010). Finally, the annotated sequences were deposited at the European Nucleotide Archive (Accession numbers CCNS01000001-CCNS01000138).

Results and discussion

The technology we used and the sequencing depth allowed us to compile a draft genome of this bacterium. The draft genome is 7,531,574 bp in size and contains 6786 genes out of which 6735 are protein coding genes, 48 tRNAs and 3 rRNAs (PRJEB6934). The size of the genome is consistent with previously sequenced genomes of *Burkholderia* strains that are on average 8Mbp in length but could span from 4 to 12 Mbp (Carlier and Eberl 2012, Xu *et al*. 2013, Carlier *et al*. 2014, Haq *et al*. 2014, Liu *et al*. 2014b). Just after we finished our analysis, a complete genome of *B. glathei* LMG14190 became available (PRJNA238428). The complete genome is 8,637,375 bp in size and contains 7661 genes encoding 7465 predicted proteins and 52 tRNAs and 4 rRNAs. These values do not differ greatly from our draft genome and show that we lacked 730 additional proteins. Comparison of our draft genome with

the complete genome revealed that these additional proteins do not encode for any new pathway but only contribute proteins to the incomplete pathways predicted in our draft genome.

The genome of *B. glathei* contains a number of pathways and transporting systems that enable this soil bacterium to adapt to different conditions, which can occur in the soil. Some relevant examples are discussed below.

Membrane transport proteins are of great importance for the cell, as they are involved in a number of functions such as uptake of substrates, maintenance of redox potentials, as well as detoxification. All these are very crucial in the soil, where cells are exposed to stress and starvation conditions and thus require a large diversity of such transport systems. We predicted the transport systems encoded in the draft genome with the online tool TransAAP (http://www.membranetransport.org/), which provided 967 predicted genes involved in membrane transport (Appendix Table 1). This is a relatively high number as it represents approximately 14% of all predicted proteins in the draft genome of *B. glathei* LMG14190 compared to around 7%, 11% and 12% of the genomes of *B. phytofirmans* PsJN, *B. terrae* BS001 and *B. rhizoxinica* HKI454, respectively (Haq *et al*. 2014). Among the classes of transporters predicted in the genome of *B. glathei* LMG1490, the ATP-binding cassette (ABC) superfamily (n=476) was the best represented, followed by the major facilitator superfamily (MFS) (n=177) and the drug/metabolite transporter (DMT) (n=27) and resistance-nodulation-cell division (RND) (n=23) superfamily. This large repertoire of transporters covers a very wide span of functions and of substrate specificities (Appendix Table 1).

In addition to this possibility to transport a large number of substrates, the *B. glathei* genome also encodes many pathways providing the ability to consume these imported nutrient sources. Among simple sugars *B. glathei* has the potential to utilize xylose, sucrose, lactose, L-rhamnose, L-arabinose, fructose, mannose and galactose. However, it can also utilize more complex polymeric carbohydrates such as glycogen, chitin and cellulose, suggesting that this organism might be an important member of the microbial community involved in decomposition of organic matter in the soil.

Degradation pathways of polyamines (e.g. 4-aminobutyrate, putrescine, urea, choline, allantoin) predicted from the genome further support the role of this bacterium in decomposition, as polyamines originate from decaying matter. The genome of *B. glathei* LMG14190 also encodes degradation pathways for aromatic compounds, which is a commonly observed feature of various *Burkholderia* strains (Pérez-Pantoja *et al*. 2012, Andreolli *et al*. 2013, Schamfuss *et al*. 2013, Xu *et al*. 2013). Beside toluene, phenol, benzoate and catechol, the complete salicylate degradation pathway is presented in the genome. Moreover, *B. glathei* has the potential to utilize alcohols such as ethanol, butanediol and glycerol. Regarding biological interactions, glycerol has been found to be one of the nutrient sources that *B. terrae* BS001 utilizes while interacting with the fungus *Lyophyllum* sp. Karst (Nazir *et al*. 2013).

Apart from a broad metabolic potential, the genome of *B. glathei* also encodes other genetic features previously described to be important for successful colonization of the soil and survival in this environment. Detoxification is an essential defence mechanism that enables microbes to cope with selective abiotic and biotic pressures. Besides multiple transporters that have a role in drug export, the genome of *B. glathei* harbours many proteins involved in heavy metal transport (Appendix Table 1). Additionally, the genome encodes a large number of peroxidases and catalases known to have a function in the defence against toxic reactive oxygen radicals.

Secretion systems are another important mechanism contributing to adaptation and survival in the environment. The role of secretion systems is transportation or translocation of effector molecules (e.g. proteins, enzymes or toxins) from inside the cell to the exterior. From the six secretion systems described in Gram-negative bacteria, the genome of *B. glathei* LMG14190 contains genes belonging to four types. Secretion systems type 2 (T2SS) and 6 (T6SS) are completely encoded, while type 4 (T4SS) and type 5 (T5SS) are incomplete. The T2SS is a two-step process dedicated to the secretion of folded and/or oligomeric exoproteins. T2SS is highly preserved in Gram-negative bacteria and involves a set of 12 to 16 different proteins (Douzi *et al*. 2012). The functions of the proteins secreted by the T2SS are extremely diverse and include toxins, surface-associated virulence factors, cytochromes and a broad range of enzymes that hydrolyse macromolecules such as polysaccharides, proteins and lipids

(Sandkvist *et al*. 1997, Horstman and Kuehn 2002, Filloux 2004, Shi *et al*. 2008). The T6SS has been recently described, yet studies have already shown that it is one of the most widespread secretion systems in Gram-negative bacteria (Mougous *et al*. 2006, Pukatzki *et al*. 2006, Boyer *et al*. 2009). This secretion system is often related with virulence of Gram-negative bacteria involved in eukaryotic interactions as well as in interactions between bacteria, as was reported for *Burkholderia* sp. (Schwarz *et al*. 2010). Importantly, the genome of *B. glathei* LMG14190 lacks the genes encoding the type three secretion system (T3SS), which was described to be important in the interaction between *B. terrae* BS001 and *Lyophyllum* sp. Karst (Warmink and van Elsas 2008) and between *B. rhizoxinica* and *Rhizopus microspores* (Lackner *et al*. 2011). Our results indicate that the T3SS is not essential for the interaction with fungi, since *B. glathei* LMG14190 was able to colonize the hyphae, migrate on them and benefit from the interaction (see below, Published results) despite the absence of this secretion system.

Finally, the genome of *B. glathei* also harbours gene clusters related with secondary metabolite biosynthesis. Using antiSMASH (Medema *et al*. 2011) for predicting homologous gene clusters, bacteriocin and terpene-like clusters were predicted as well as clusters with unknown function and four nonribosomal peptide synthesis (NRPS) clusters, involved in pyoverdine production. In addition to these above mentioned secondary metabolites, the genome harbours genes involved in the synthesis of the exopolysaccharide cepacian. Cepacian is produced by most *Burkholderia* species. It is involved in the establishment of biological interactions and plays an important role in the resistance to different stresses (Ferreira *et al*. 2011).

The genetic features that are encoded in the genome of *B. glathei* could be a reason why this species is so largely distributed in the soil environment (Stopnisek *et al*. 2014): *B. glathei* is able not only to successfully respond to stressful environmental conditions, it also has the potential to compete and interact with other components of the soil microbiota.

Published results

Manuscript submitted in October 2014 to the ISME Journal.

Molecular mechanisms underlying the close association between soil **Burkholderia** *and fungi*

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Abstract

Species belonging to the genus *Burkholderia* have been repeatedly reported to be associated with fungi but the extent and specificity of these associations in soils is so far unknown. Moreover, the molecular basis of the interaction and its influence on the physiology of *Burkholderia* is poorly understood. To assess whether associations between *Burkholderia* and fungi are widespread in soils, we performed a global co-occurrence analysis in an intercontinental soil sample collection. This revealed that members of the *Burkholderia* genus significantly co-occurred with a wide range of fungal species. To analyse the molecular basis of the interaction, we selected two model fungi frequently co-occurring with *Burkholderia*, *Alternaria alternata* and *Fusarium solani*, and analysed the proteome changes caused by cultivation with either fungus in the widespread soil inhabitant *B. glathei*, whose genome we sequenced. Co-cultivation with both fungi led to very similar changes in the *B. glathei* proteome. Our results indicate that *B. glathei* significantly benefits from the interaction, which is exemplified by a lower abundance of several starvation factors that were highly expressed in pure culture. However, co-cultivation also gave rise to stress factors, as indicated by the increased expression of multidrug efflux pumps and proteins involved in oxidative stress response. Our data suggest that the ability of *Burkholderia* to establish a close association with fungi mainly lies in the capacities to utilize fungal-secreted metabolites and to overcome fungal defense mechanisms. This work indicates that beneficial interactions with fungi might contribute to the survival strategy of *Burkholderia* species in a nutrient limited environment such as acidic soil.

Introduction

Members of the genus *Burkholderia* belong to the class β-*Proteobacteria* and are widely distributed in the environment. *Burkholderia* species can on the one hand cause severe damage to cystic fibrosis patients or to plants and on the other hand promote plant growth, or take part in remediation processes as effective pollutant removers (Vial, 2007). As common soil microbes they are also involved in biological interactions with different plants (Elliott et al., 2009; Carlier and Eberl, 2012), invertebrates (Kikuchi *et al*., 2005) and fungi (Warmink *et al*., 2009; Warmink *et al*., 2011; Nazir *et al*., 2012; Uroz *et al*., 2012; Scherlach *et al*., 2013). Since *Burkholderia* are mostly found in acidic soils (Stopnisek *et al*., 2014), interactions with fungi might be of particular relevance as fungi also favour acidic environments and both types of organisms are thus likely to inhabit similar niches. From in vitro studies, we know that *Burkholderia* can form either antagonistic or mutualistic interactions with fungi. Antagonistic behaviour of *Burkholderia* species is well described and is largely due to production of multiple antifungal compounds (Hammer et al., 1999; Lewenza and Sokol, 2001; Partida-Martinez and Hertweck, 2007; Schmidt *et al*., 2009; Chen *et al*., 2013) showing effects towards a number of phytopathogenic fungi (Quan *et al*., 2006; Palumbo *et al*., 2007; Kilani-Feki *et al*., 2011; Groenhagen *et al*., 2013). Interestingly, most of these compounds are produced by species belonging to the pathogenic group of *Burkholderia* that also includes the *Burkholderia cenocepacia* complex (Bcc). In contrast, it has been reported that many environmental *Burkholderia* strains exhibit beneficial effect with fungi, suggesting symbiotic and/or mutualistic interactions. Benefits from such interactions were mainly studied in a model system comprised of *B. terrae* and *Lyophyllum* sp. This bacterium is able to colonize the hyphae of Lyophyllum sp., which allows them to translocate and disperse (Warmink *et al*., 2011). Additionally, *Lyophyllum* sp. alleviates pH pressure in acidic soils and with that enhances survival of associated bacteria (Nazir *et al*., 2010a). Furthermore, *Burkholderia* strains have been shown to have the capacity to utilize several fungal exudates as nutrient source (Warmink *et al*., 2009; Nazir *et al*., 2010b; Nazir *et al*., 2013). Along these lines, Drigo *et al*. (2013) reported that *Burkholderia* strains were among the main consumers of C sources released from arbuscular mycorrhizal fungi in a rhizosphere community incubated under elevated CO2. In addition to the supply of nutrients and the facilitated

transportation, it has been suggested that the presence of fungi could be essential for survival of *Burkholderia* in soils: *Burkholderia* strains were found to be unable to re-colonize the native soil they had been isolated from when this soil was sterilized prior to re-inoculation (Nazir *et al*., 2010a; Warmink *et al*., 2011). Yet, when fungi were re-inoculated to the sterile system together with the *Burkholderia* strains, these were able to maintain stable population levels in the re-colonized soil (Nazir *et al*., 2010a).

Despite these reports, which indicate that *Burkholderia* benefits from their fungal partners in soils, two main questions remain unanswered: i) how widespread are such associations in soils and how specific are they, and ii) what is the molecular basis of these interactions and what are the benefits for the bacteria?

To answer these questions, we designed a comprehensive study in which co-occurrence network analysis, cultivation based methods and proteomics were used. Co-occurrence network analysis was carried out on a collection of soils from a transcontinental study to gain information on the extent, the specificity and the distribution of such interactions in the soil. Model *Burkholderia* strains were tested further for their ability to translocate and disperse in the presence of fungi. Finally, the widespread soil inhabitant *B. glathei* was grown alone or in the presence of either *Fusarium solani* or *Alternaria alternata* and the proteins expressed in *B. glathei* under each situation were compared to shed light on the molecular and physiological basis of the interaction.

Methods

Co-occurrence analysis

We analyzed the microbial communities of 266 soil samples from the Nutrient Network (NutNet) globally distributed experiment (Borer *et al*., 2014). 37,393 ITS fungal OTUs and 223,693 16S rRNA bacterial OTUs were included into the analysis. Co-occurrence patterns between bacterial and fungal communities were tested using Spearman's rank correlations between OTUs that occurred in at least 20% of the samples and had a rho > 0.5 and p-value < 0.01 adjusted using the false discovery rate (FDR) method (Barberan *et al*., 2011).

Monitoring the interactions between fungi and *Burkholderia* sp.

To study the interactions between *Burkholderia* sp. and fungi, we used a combination of cultivation methods and microscopy. To minimize the effect of the growth medium on *Burkholderia* growth, we used only dissolved agar in distilled water (15 g l-1). Fungi used in this experiment belonged to the species *Alternaria alternata*, *Fusarium solani*, *Rhizoctonia solani* and *Lyophyllum* sp. Karst, while *Burkholderia* species applied were *B. glathei*, *B. hospita*, *B. fungorum* and *B. caledonica* (Table 1).

To visualize the interaction with fungi under the fluorescent microscope, all *Burkholderia* strains were tagged with either the green fluorescent protein (GFP) or the dsRED protein using the electroporation protocol described by Choi *et al*. (2006). In short, overnight cultures of *Burkholderia* strains were washed twice in 0.3M sucrose and subsequently electroporated with the plasmid pBBR1MCS-2-gfp mut3 Kmr or pin62: DsRed Cmr. Electroporated cells were transferred on PIA and LB plates with the corresponding antibiotics (50 mg/l). To follow the interactions, fungi were inoculated on water agar plates or Luria-Bertani (LB) agar at least 3 days before spotting the bacterial strains (tagged or untagged) on the mycelium. For that overnight liquid *Burkholderia* cultures were prepared, washed three times in 0.9% NaCl solution, OD600 was adjusted to 0.1 and 4 drops of 2 µl were spotted on the fungal mycelium pre-grown for three days (see Figure 2). Plates were incubated for up to 10 days in the dark and growth of *Burkholderia* and interactions with fungi were followed daily under the fluorescence microscope (Leica M165 FC) and binocular

(Leica DM6000 B). Additionally, dispersal and attachment of *Burkholderia* strains on fungal hyphae was tested. For that, a sterile iron ring was placed in the middle of the plate, which served as physical barrier for bacterial cells, prior to pouring the plate. Both sides were filled up to 5mm below the edge of the iron circle. Selected fungi and *Burkholderia* strains were cultivated in the inner side of the ring and the interactions were followed as previously. In this system, only bacterial cells that were able to attach and/or disperse would be detected on the outer side of the iron ring.

Enrichment of *Burkholderia* cells from fungi and protein extraction

For proteomic analyses, *B. glathei* LMG14190 and two fungi, *A. alternata* and *F. solani*, were used. Co-cultures with *A. alternata (*AB) and *F. solani* (FB) as well as the *B. glathei* alone (control) were grown in triplicates as described above with some modification. As growth medium 1/3 MEA (Difco, USA) supplemented with 7 g agar l-1 medium was used. For easier collection of organisms from the plates a cellophane membrane was used (Bio Rad, USA, 165-0963-MSDS). Plates were incubated for up to 10 days at room temperature. Thereafter, the biomass was collected with an inoculation loop, and samples were treated as described by Carlier and Eberl (2012) with some modifications. In short, samples were homogenized in phosphate-buffered saline solution (PBS) with a pestle, debris were pelleted at 1000 g for 3 min in a benchtop centrifuge and the top layer was transferred to 50% Percoll (Sigma-Aldrich, USA) for separation of bacterial and fungal cells. After ultracentrifugation at 15000x g for 3 h, fractions were collected and analysed by phase contrast light microscopy (Leica DM6000 B) and fractions containing predominantly bacteria were pooled and washed 2 times in PBS. To extract proteins from cells in pooled fractions a bead-beating protocol was used. Fractions were transferred into 2 ml tubes (Sarstedt, Germany) with 0.25 ml of 0.1 mm glass beads (BioSpec Products, USA) and placed into FastPrep®-24 lyser (MP Biomedicals, USA). Eight rounds of 30 sec lysis of 6.5 m/s followed by 5 min incubation on ice were used to extract proteins in concentrations from 2 to 8 mg ml-1.

Protein sample preparation and mass spectrometry analysis

25 μg of cytosolic protein extract (determined using Roti®-Nanoquant, Roth, Karlsruhe, Germany) were resolved on 1D SDS-PAGE, lanes were cut in equidistant pieces and in-gel digested with trypsin as recently described (Grube *et al*., 2014). Peptide mixtures were separated by RP chromatography using an EASYnLC 1000 (Thermo Fisher Scientific, Waltham, MA, USA) with self-packed columns. Peptides were loaded and desalted on the separating column following resolution with a binary non-linear 76 min-gradient from 5 – 75 % ACN in 0.1 % acetic acid at a constant flow rate of 300 nL/min. The LC system was coupled online to an Orbitrap Elite equipped with a nanoelectrospray ion source (Thermo Fisher Scientific, Waltham, MA, USA) performing MS and MS/MS experiments of the eluted peptides. Survey scans were recorded in m/z range from 300 – 1700 with a resolution of 60,000 and with lock mass option enabled. MS/MS experiments were performed for the 20 most intensive precursor ions as determined in the survey scan excluding unassigned charge states or singly charged ions from the MS/MS experiments in the Linear ion trap.

Protein identification, relative protein quantification and statistical data analysis

Proteins were identified by searching all MS/MS spectra against a database containing protein sequences of *Burkholderia glathei* LMG14190, *Fusarium solani* and *Alternaria alternata* (version 13-Dec-17) with added laboratory contaminants using SorcererTM-SEQUEST® (Sequest v. 27 rev. 11, Thermo Scientific) including Scaffold_4.0.5 (Proteome Software, Portland, OR, USA). SEQUEST® was searched with a parent ion tolerance of 10 ppm and a fragment ion mass tolerance of 1.00 Da. Up to two missed tryptic cleavages were allowed. Methionine oxidation (+15.99492 Da) was set as variable modification. For protein identification, a stringent SEQUEST® filter for peptides was used (Xcorr versus charge state: 2,2, 3,3 and 3,8 for doubly, triply and quadruply charged peptides and deltaCn value greater than 0.10) and at least two peptides per proteins were required. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. The protein FDR, based on a decoy database, was 0.4 %. Relative quantification was based on spectral counting of exclusive spectra using normalized spectral abundance factors (Zhang *et al*., 2010).

Statistical analysis using MeV v4.8.1 (Saeed *et al*., 2003) was done for proteins that were present in at least two out of three biological replicates. Hierarchical clustering and student's t-test of z-transformed normalized exclusive spectra was performed with the following settings: unequal group variances were assumed (Welch approximation), p-values based on permutation (1000) with p=0.05, significance determined by standard bonferroni correction. To account for biological relevance only proteins were considered for further analysis that showed at least two-fold changes in addition to statistical significance. Furthermore, so-called "off/on" proteins needed to be detected in all replicates of one treatment and absent from all replicates of another treatment.

Functional prediction and assignment of proteins to cluster of orthologous groups (COG) and TIGRFAMs, respectively, was accomplished by the in-house developed analysis pipeline "Prophane 2.0" (http://www.prophane.de; (Schneider *et al*., 2011)). Voronoi treemaps were generated using Paver (Decodon, Greifswald, Germany, http://www.decodon.com/).

Burkholderia glathei LMG14190 genome sequencing

To allow better analysis of the proteomic data, we sequenced the genome of *B. glathei* LMG14190. To this end, high molecular mass DNA was extracted following a standard protocol (Wilson, 2001). Paired-end insert libraries were constructed at the Functional Genomics Center Zurich (FGCZ) using the Nextera XT kit (Illumina, San Diego, CA, USA). Sequencing of the 2x250 bp inserts was performed on the Illumina MiSeq platform at the FGCZ. The sequencing reads were assembled using the SPADES v2.5 software (Nurk *et al*., 2013). The resulting 138 contigs were examined and edited for misassemblies in GAP5 (Bonfield and Whitwham, 2010). Ribosomal RNA and tRNA genes were predicted using RNAmmer and tRNAscan (Schattner *et al*., 2005; Lagesen *et al*., 2007). The predicted RNA genes were masked using the maskfeat utility of the EMBOSS software package (Rice *et al*., 2000) prior to protein-coding gene finding using the Prodigal software (Hyatt *et al*., 2010). The annotated contigs, containing CDS and RNA genes predictions were submitted to the RAST online service for functional annotation (Aziz *et al*., 2008). The annotations were curated using the Artemis software suite (Carver *et al*., 2008) and the annotated

sequences were deposited at the European Nucleotide Archive (Accession numbers CCNS01000001-CCNS01000138).

Results and discussion

Burkholderia co-occur with fungi in soils

Since the relative abundance of soil *Burkholderia* populations was shown to be increased in acidic soils (Stopnisek *et al*., 2014), which are also known to be enriched for fungi, we wondered whether members of the *Burkholderia* genus would show a co-occurrence pattern with specific fungal populations. To gain information about the frequency of fungi co-occurring with the genus *Burkholderia* as well as with other bacterial genera we performed a co-occurrence analysis on sequences obtained from a trans-continental collection of soil samples. Evidence for strict co-occurrence might indicate that besides sharing the same niche, mutualistic interactions between organisms exist. The analysis supported our hypothesis that members of the genus *Burkholderia* significantly co-occur with fungi as OTUs from the genus *Burkholderia* were among the ten OTUs most frequently associated with fungi (Fig. 1). Interestingly the analysis showed high co-occurrence frequency with multiple fungal taxa, suggesting broad and frequent associations between fungi and *Burkholderia* in soils (Supplementary table S1). Apart from *Burkholderia*, other bacterial genera also showed high co-occurrence with fungi. Whether these patterns reflect an intimate interaction with the fungi or are a result from their shared preference for low pH or other environmental parameters remains to be investigated. For *Acidobacteria* and *Verrucomicrobia*, two phyla containing OTUs that showed high co-occurrence with fungi, low pH-dependency has been demonstrated but to our knowledge there is no evidence for beneficial interactions between members of those phyla and fungi. In contrast, while the genus Burkholderia also shows strong preference for acidic soils (Stopnisek *et al*., 2014), mounting evidence suggests that many species of this genus engage in interactions with fungi (Warmink *et al*., 2011; Nazir *et al*., 2012; Uroz *et al*., 2012). Thus we speculate that the strong co-occurrence of *Burkholderia* and fungi indicates that the two organisms interact with each other in the soil, rather than just reflecting a shared niche preference.

Burkholderia sp. attach, translocate and disperse on fungal hyphae

To test the specificity of the interactions and the ability of *Burkholderia* to attach, translocate or disperse on hyphae of different fungal species an in vitro experiment was designed. To this end, four *Burkholderia* strains and four fungi chosen among those taxa significantly co-occurring with *Burkholderia* (Table 1) were co-inoculated and their growth monitored. The ability to interact was followed in Petri dishes where selected fungi and *Burkholderia* strains were grown in dual cultures. To verify whether the four *Burkholderia* strains could use fungal hyphae as a mean of translocation and dispersal, we used an iron ring to separate a Petri dish in two compartments (Fig. 2). In the inner one, the fungus and the bacteria were inoculated together. Only those bacterial cells able to attach to the hyphae would be able to cross the iron ring to reach the outer compartment. Water agar was used as a mean to study putative beneficial effects of fungi on *Burkholderia* cells, as they were not able to grow on water agar. In addition, we used LB agar plates to investigate whether *Burkholderia* interacts with the fungus or grows independently on this medium. Interestingly, even when the rich LB medium was used in the outer compartment, *Burkholderia* strains were mainly found in close vicinity of fungal hyphae, suggesting that the benefits provided by the fungi were not restricted to transportation, but included other goods that caused bacteria to stay on the hyphae rather than spread on the rich agar medium. Our experiments showed that i) for all four tested *Burkholderia* strains, growth on the hyphae was favoured over growth on the medium (Fig. 2) and ii) all tested *Burkholderia* strains were able to cross the iron ring and be translocated with the hyphae of all fungi tested, except for *Lyophyllum* sp. Karst. Under the conditions used, the mycelium of *Lyophyllum* showed hydrophobicity, thus inhibiting the formation of a water film around the hyphae, which might be necessary for bacterial attachment and dispersal (Pion *et al*., 2013a). However, Warmink *et al*. (2011) showed that *B. terrae* BS001 was able to migrate together with *Lyophyllum* sp. Karst in a soil microcosm study, suggesting that the physiology of this fungus may change when growing in soil. Nazir *et al*. (2012) tested 19 *Burkholderia* strains for their migration capacity on this fungus. All strains were able to co-migrate, but their abilities differed depending on the soils used, confirming the importance of the environment in this type of interaction. No visible beneficial or deleterious effect of the bacteria on mycelial growth or morphology was observed. This absence of

deleterious effects is in line with the observations of Pion *et al*. (2013b), who monitored the association between *P. putida* KT2440 and the fungus *Morchella crassipes*. However, in contrast to our results, bacterial inoculation triggered visible physiological changes in *M. crassipes*, as revealed by increased sclerotia formation.

Global analyses to elucidate the molecular basis of *Burkholderia*-fungi interactions

We used a proteomic approach in order to characterize the molecular nature of the interaction between *Burkholderia* strains and fungi. Among the fungal OTUs that co-occurred with *Burkholderia*, those belonging to the genera *Alternaria* sp. and *Fusarium* sp. were among the most frequently detected ones in our analysis. This is why we selected *A. alternata* and *F. solani* as model species. As a representative of the genus *Burkholderia*, *B. glathei* LMG14190 (T) (Vandamme *et al*., 1997) was chosen for these interaction studies, since members of this species were not only the most frequently detected in our soil survey (Stopnisek *et al*., 2014), but have also been demonstrated to interact with fungi (Koele *et al*., 2009).

Genetic features of *B. glathei* LGM14190 possibly involved in interactions with fungi

To obtain insights into the reasons for the ubiquitous distribution of *B. glathei* in soils, as well as for better interpretation of proteomics data, we sequenced the genome of *B. glathei* LMG14190, which was not available at the time of our analyses. The draft genome is 7,531,574 bp in size, which is consistent with already sequenced strains of the genus *Burkholderia* (Xu *et al*., 2013; Carlier *et al*., 2014; Liu *et al*., 2014). Annotation of the draft genome predicted 6786 genes out of which 6735 are predicted to code for proteins, 48 for tRNAs and 3 for rRNAs (PRJEB6934). The genome of *B. glathei* LMG14190 encodes a large number of genes involved in transport and utilization of various carbon sources. In addition to the utilization of simple sugars, *B. glathei* LMG14190 has the genetic potential to degrade polymeric carbohydrates such as chitin, cellulose, glycogen, and for the degradation and utilization of alcohols, including ethylene glycol, ethanol and butanediol. *B. glathei* LMG14190 contains genes coding for enzymes involved in the degradation of glycerol, which is predicted to be one of the C sources fungi secrete and that would be available to interacting

Burkholderia strains, as previously postulated for *B. terrae* BS001 (Nazir *et al.*, 2013).

Additionally, fungi produce a number of aromatic compounds that could represent another source of nutrients (Gutiérrez *et al.*, 1994). Degradation of aromatic compounds is a commonly observed feature of various *Burkholderia* strains (Pérez-Pantoja *et al.*, 2012; Andreolli *et al.*, 2013; Schamfuss *et al.*, 2013; Xu *et al.*, 2013) and corresponding genes were also identified in the genome of *B. glathei* LMG14190. Beside pathways for the utilization of toluene, phenolic, benzoate and catechol compounds, the genome also encodes the complete salicylate degradation pathway. However, multiple aromatic compounds have also antibacterial and antifungal effects and thus the capacity to degrade such substances could also serve as a protection mechanism for both bacterial cells and their interacting partners.

The type three secretion system (T3SS) has been described to be essential in the interaction between *B. terrae* BS001 and *Lyophyllum* sp. Karst (Warmink and van Elsas, 2008). However, homologous genes could not be detected in the genome of *B. glathei* LMG14190. We also tested for the presence of the hrcR gene, a highly conserved component of the type three secretion systems (T3SS) (Warmink and van Elsas, 2008). Surprisingly, the hrcR gene could only be amplified from one of the strains tested, *B. hospita*, while it was not detected in the three other strains tested (*B. glathei*, *B. fungorum*, *B. phytofirmans*) (data not shown). This suggests that T3SS is not essential for the interaction with the fungi used in the present study. Nonetheless the genome of *B. glathei* LMG14190 harbours genes that have been previously described to play a role in biological interactions, such as those involved in the synthesis of the exopolysaccharide cepacian (BGLT00414-BGLT00424), which is produced by most *Burkholderia* species and is important for interactions with other organisms as well as for resistance to different stresses (Ferreira *et al.*, 2011).

Global changes in the proteome of *B. glathei* when co-cultivated with fungi

A total of 2166 unique *B. glathei* LMG14190 proteins and only 3 fungal proteins were detected in the present study, indicating an efficient enrichment of bacterial cells from

the co-cultures and representing approximately 32% of the theoretical proteome predicted based on the *B. glathei* genome sequence (Supplementary table S2). After applying stringent filters and considering only proteins identified in at least two out of three biological replicates, 1411 proteins remained, the expression of which could be semi-quantitatively assessed by spectral counting (Supplementary table S3). Among those, 815 (almost 60%) were identified in both, single and co-cultures, while 262, 45 and 27 proteins were found to be exclusively expressed during single culturing or co-cultivation with *A. alternata* and *F. solani*, respectively (Fig. 3). Overall, 696 were differentially expressed (expression change of more than 2-fold) during co-cultivation compared to the single cultures with the majority of proteins expressed in lower amounts in the presence of either of the fungi.

Most of the identified proteins were assigned to functions in production and conversion of energy, as well as in transport and metabolism of carbohydrates and amino acids (Supplementary table S2). This corresponds to the high proportion of predicted genes with the same function in the genome of *B. glathei* LMG14190 (approximately 20% of the whole genome). In contrast, functional classes such as transcription, defence and signalling were under-represented in the *B. glathei* proteome when considering their contribution to the total number of predicted genes (Supplementary table S2).

To shed light on the molecular mechanisms involved in bacterial-fungal interactions, we focused mainly on the *B. glathei* proteins whose abundance changed significantly (more than 2-fold) during fungal co-cultivation. As shown in Fig. 4 and 5 co-cultivation affected protein expression across all functional categories. Notably, a particular high percentage of differentially expressed proteins were involved in cell motility and translation (Fig. 4, Fig. 5). The putative relevance of the observed protein expression changes during co-cultivation to the biological interaction between *B. glathei* and fungi are highlighted below.

Attenuation of starvation and various stress responses in *B. glathei* when co-cultivated with fungi

B. glathei grown in the absence of fungi were limited to the nutrients provided by the growth medium (1/3 MEA) and this resulted in the expression of many transcriptional regulators related to starvation and stress response. Interestingly, in co-cultivations the expression rates of most of these regulators and stress factors were significantly reduced suggesting multiple beneficial effects of fungi on *B. glathei* (Supplementary table S3). From a total of 243 predicted proteins in signal transduction mechanisms, 33 and 30 were present in co-cultivations with *A. alternata* (AB) and *F. solani* (FB), respectively, with 27 of them shared between the co-cultivations (Supplementary table S3), suggesting similar response and regulation of the metabolic pathways of *B. glathei* when growing with both fungi. Most of these proteins were factors related to stress or starvation conditions linked to important nutrients such as nitrogen, phosphate and carbon, which were all relieved upon co-cultivation. For instance, the attenuation of nitrogen (N) starvation in both co-cultivations was supported by the decrease of the nitrogen regulation protein NRI (BGLT03875) and the glutamine synthetase (BGLT02655) when compared with single cultivation. The nitrogen regulation protein NRI is the major activator of nitrogen-controlled genes such as the glutamine synthetase (Reitzer and Magasanik, 1985; Reitzer *et al*., 1989). It has been shown that nitrogen starvation in *E. coli* leads also to an increase of proteins involved in amino acid and polyamine degradation (Zimmer *et al*., 2000). Decreased expression of various amino acid transport systems as well as of amino acid and polyamines degradation pathways in *B. glathei* in co-cultivations additionally supports the idea that the fungi alleviate the N starvation response (Fig. 4, 5). Even though most of the pathways and transport systems show lower abundances compared to the single culture, subunits of histidine ABC transporters (BGLT00146, BGLT01156) are significantly increased (2- to 3-fold) in *B. glathei* co-cultivated with the fungi. Interestingly, histidine could act as N source provided by fungi to *B. glathei* cells, since many enzymes of the histidine degradation pathway, such as histidine ammonia-lyase (BGLT04159), urocanate hydratase (BGLT03664) and N-formylglutamate deformylase (BGLT04163), were detected (Supplementary table S3).

Beside N starvation, phosphate (P) starvation seemed alleviated in co-cultivation when compared with the control, as indicated by a significant decrease or absence of proteins such as phosphate starvation-inducible protein (BGLT03521) and glycerol-3-phosphate uptake system (BGLT04691, BGLT05290, BGLT00157) in the co-cultivation experiment. The phosphate starvation-inducible protein PhoH is part of phosphate regulon in *E. coli* and its expression is induced by phosphate starvation (Metcalf *et al.*, 1990). The UgpBAEC glycerol-3-phosphate uptake system is a member of the ABC superfamily. Expression of proteins associated with this system was among the most strongly decreased in the co-cultivation experiments. In *E. coli*, glycerol-3-phosphate can be used as C and/or P source and is also an essential intermediate in phospholipid biosynthesis (Schweizer *et al.*, 1982; Boos, 1998). Glycerol-3-phosphate that is transported through the Ugp system is an insufficient carbon source for *E. coli*, however it can sustain growth as a sole P source (Boos, 1998). Thus, the Ugp system is used for scavenging phosphate-containing compounds in *E. coli*, which may also be true in *Burkholderia*. In contrast to most proteins that were significantly decreased in co-cultivations, the acid phosphatase (BGLT02502) was slightly increased during co-cultivation with *F. solani*. This enzyme is known to be induced by P starvation in *B. cenocepacia* J2315 and is secreted to the environment, suggesting a role in P acquisition (Yeung *et al.*, 2009). Increase of phosphate solubilisation by *Burkholderia* sp. and other bacteria in the ectomycorrhizosphere has been described by Calvaruso *et al.* (2007), suggesting that phosphate acquisition is carried out independently by each of the partners.

The limited amount of carbon contained in the growth medium could explain the detection of proteins indicative of C starvation in the pure culture control. However, even though the same medium was used in the co-cultivation experiments, most of the C starvation proteins were absent or strongly decreased in the presence of either of the fungi. For instance the Stringent starvation protein A, SspA (BGLT04645) was strongly decreased in the co-cultivation experiments. This protein is known to be implicated in survival during nutrient starvation and prolonged stationary phase. In *E. coli*, SspA expression is induced by starvation for glucose, nitrogen, phosphate, amino acids as well as by a decreased growth rate (Williams *et al.*, 1994). Besides SspA, a protein that is more specifically expressed during C starvation was also detected: the

carbon starvation protein A, CstA (BGLT04027), was significantly decreased when *F. solani* was present, while no change was detected in co-cultivation with *A. alternata*. It has been shown in *E. coli* and *Campylobacter jejuni* that overexpression of *cstA* leads to the synthesis of starvation-inducible proteins and that it is involved in peptide uptake and utilization (Matin, 1991; Schultz and Matin, 1991; Rasmussen *et al.*, 2013).

The decreased amount of SspA and other C starvation proteins during co-cultivation suggests a preference of *B. glathei* towards the utilization of fungal exudates over the C source present in the growth medium. This is supported also by the decreased levels of the maltose ABC transporter (BGLT01992) in co-cultivations, which is responsible for uptake of maltose, the main C-source in the growth medium (MEA). Additionally to the decreased amount of the maltose transporter, a xylose ABC transporter (BGLT06434) was also strongly decreased in co-cultivation. D-xylose is the most abundant sugar in nature after glucose, and it can be utilized by *E. coli* as a sole carbon source and metabolized through the pentose phosphate pathway (Song and Park, 1998). That xylose was actually used when *B. glathei* grew alone on MEA is suggested by the expression of xylose isomerase (BGLT06433), which catalyzes the first reaction in the catabolism of D-xylose (Schellenberg *et al.*, 1984) and which was not present in the co-cultivations with either fungus (Supplementary table S3).

These results suggest that *B. glathei* may preferentially utilize fungal exudates in the co-cultivation experiments. However, we could not unambiguously identify the primary C source of *B. glathei* in the exudates. A likely candidate appears to be ribose, as the amounts of proteins involved in ribose uptake (BGLT04604, BGLT04606 and BGLT05567) were strongly increased in the co-cultivation experiments.

Increased numbers of proteins involved in detoxification processes in the presence of both fungi.

In contrast to the previously mentioned positive effects of fungi on nutrient starvation alleviation, proteins indicating stress related effects were also detected upon their

presence. Increase of various catalases (BGLT01132, BGLT04737) and peroxidases (BGLT04737) in co-cultivations suggests that co-cultivation induced oxidative stress (Supplementary table S3). In addition, increased expression of 5-methyltetrahydropteroyltriglutamate homocysteine methyltransferase, MetE (BGLT01007) in co-cultivation with *A. alternata* also suggests oxidative stress, since expression of MetE is very high in *E. coli* when cells are exposed to oxidative stress (Hondorp and Matthews, 2004). Antibacterial compounds that are released by the fungi could cause oxidative stress. Production and secretion of such compounds has been reported for the two fungi used in this study (Ammar *et al*., 1979; Hellwig *et al*., 2002; Deshmukh *et al*., 2014; Soltani and Hosseyni Moghaddam, 2014). Increase of the RND (Resistance Nodulation Division) family of efflux pumps (BGLT02584, BGLT02585, BGLT02586, BGLT04622, BGLT05365) in co-cultivations further supports the idea of fungal release of antibacterial agents. The detected proteins show high homology to members of the NodT family of outer membrane transport proteins from the genus *Rhizobium* (Rivilla *et al*., 1995) and the CmeABC multidrug efflux system of *Campylobacter jejuni* (Lin *et al*., 2002), which are involved in the export of a wide range of drugs. In addition to drug resistance, RND pumps play important role in biological interactions such as nodulation, colonization and host persistence (Piddock, 2006). In *B. cenocepacia*, RND efflux pumps also influence phenotypic traits involved in pathogenesis, such as motility and chemotaxis (Bazzini *et al*., 2011). In addition to these detoxification systems, other proteins with a putative function in detoxification were found to be increased in co-cultivations: the proteins YagS (BGLT01120), YagT (BGLT01121) and YagR (BGLT00865), which are part of the yagTSRQ operon and were significantly increased in both co-cultivation setups (Supplementary table S3). This operon was shown to encode a periplasmatic aldehyde oxidoreductase that oxidizes a broad spectrum of aldehydes to their respective acids, thereby contributing to the detoxification of the cells (Neumann *et al*., 2009).

RND efflux pumps were not the only proteins that were increased and that are associated with cell envelope biogenesis and outer membrane structures. Many outer membrane porines and exporters (BGLT00225, BGLT02300, BGLT00513, and BGLT04032) were highly increased. Although the functions of most of these proteins are so far unknown, it is worth noting that in a recent transcriptomic study, in which

B. cenocepacia J2315 was exposed to various stresses, most of the homologous proteins were increased under nutrient starvation and low oxygen conditions or when exposed to ROS stress (Sass *et al.*, 2013). Thus, the expression and increase of membrane-associated proteins observed in co-cultivation with fungi might be due to stresses caused by antibacterial agents produced by the fungi. However, changes in membrane composition could also originate from other stresses such as osmotic stress. The primary response to osmotic stress in most bacteria is the uptake of potassium (Paul, 2013), which was also detected in our proteomics approach. The genome of *B. glathei* encodes two homologous potassium uptake systems, one of which (BGLT02399, BGLT02400 and BGLT0240) was expressed upon co-cultivation with *A. alternata* and the other one (BGLT06537, BGLT06538 and BGLT06539) upon co-cultivation with *F. solani* (Supplementary table S3).

B. glathei proteins involved in motility are not expressed in fungal co-culture

Using fungal hyphae for dispersal has been shown in previous studies to be advantageous in an environment such as soil where heterogeneity of soil particles and the lack of water represent major obstacles. It was even hypothesised that the benefit of such dispersal explains the maintenance and persistence of flagellar motility in water unsaturated ecosystems (Pion *et al.*, 2013a). In our in vitro experiments, dispersal of *Burkholderia* strains on fungal hyphae was observed in spite of the fact that many proteins involved in motility were undetected in the presence of the two fungi, while they were expressed in the control (Supplementary table S3, Fig 4, 5). This might indicate that bacterial cells encountering a fungal hypha cease to invest resources into their own motility apparatus and rather rely on the fungus for transport. Loss of flagellar proteins, as well as rearrangements in cell envelope/wall structures in the co-cultivation experiments could also indicate biofilm formation. Previous studies have suggested that motility is not required for dispersal of *Burkholderia* strains on fungal hyphae, but is dependent on biofilm formation on the hyphae tips (2009). While our microscope observations revealed that biofilm formation of *Burkholderia* strains occurred around the fungal hyphae, we could not detect bacterial biofilms on the tips of the hyphae. Interestingly, in an attempt to study the effect of flagella on dispersal and fitness of *P. putida* KT2440, Pion *et al.* (2013a) concluded that flagella

mutants only experienced dispersal and fitness disadvantages compared with the wild type under water unsaturated conditions.

Conclusion

Evidence has emerged over the past years that *Burkholderia* sp. is often associated with fungi in natural soils (Warmink *et al*., 2009; Lepleux *et al*., 2012; Nazir *et al*., 2012). The co-occurrence analysis presented in this study fully supports this notion and extended our knowledge about the ability of member of the genus *Burkholderia* to form associations with a broad range of fungal taxa. *Burkholderia* glathei, *B. terrae*, *B. fungorum* and *B. phytofirmans* were tested for their capacity to interact and disperse with the fungi *A. alternata*, *F. solani*, *R. solani* and *Lyophyllum* sp. Karst. The maintenance and translocation of *Burkholderia* cells grown with the fungi showed that these interactions occurred broadly, suggesting that they might also occur in the environment. To better understand the molecular basis of the interaction, we used a proteomics approach to analyse the influence of two fungal strains on *B. glathei*. Previous reports indicated that the interactions might be beneficial for the bacterial partner, yet knowledge of the nature of these benefits is scarce (Nazir *et al*., 2010a; Warmink *et al*., 2011; Nazir *et al*., 2013). Our global analysis revealed that in a nutrient-limited medium *B. glathei* was able to use multiple substrates provided by the fungi, which attenuated the starvation response observed for cultures grown in the absence of a fungus (Fig. 6). However, *B. glathei* encountered new stresses in the presence of the fungi, as seen by the differential expression of various defence and tolerance mechanisms. These functions are likely of great importance for the successful colonization and persistence of *Burkholderia* sp. on fungal hyphae. Combining our results with the fact that soil is a nutrient limited environment we conclude that the benefits that *Burkholderia* gain from the interaction with fungi outweigh the costs involved in the co-existence, e.g. expression of functions required for detoxification and thus represents a highly successful strategy to survive in nutrient limited environment.

Acknowledgements

We are grateful to Sandra Maass and Andreas Otto for helping with protein identification by mass spectrometry. Financial support of the Swiss National Science Foundation (grant 31003A-130089 to LW) is gratefully acknowledged. AB is supported by a James S. McDonnell (JSMF) Postdoctoral Fellowship.

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Tables, figures and legends

Table 1. List of bacterial and fungal strains used in the present study with their application and references.

Bacterial and fungal strains used in this study	Application	References
Burkholderia fungorum LMG16225	Interaction studies	Coenye *et al*. (2001)
Burkholderia glathei LMG14190	Interaction studies, genome sequencing, proteomics	Vandamme *et al*. (1997)
Burkholderia hospital LMG20598	Interaction studies	Goris *et al*. (2002)
Burkholderia phytofirmans LMG22487	Interaction studies	Sessitsch *et al*. (2005)
Alternaria alternata	Interaction studies, proteomics	Phytopathology group of the Institute of Plant Sciences (Federal Institute of Technology, Zurich, Switzerland)
Fusarium solani	Interaction studies, proteomics	
Rhizoctoni solani	Interaction studies	
Lyophyllum sp. Karst DSM 2979	Interaction studies	Leibnitz Institute DSMZ

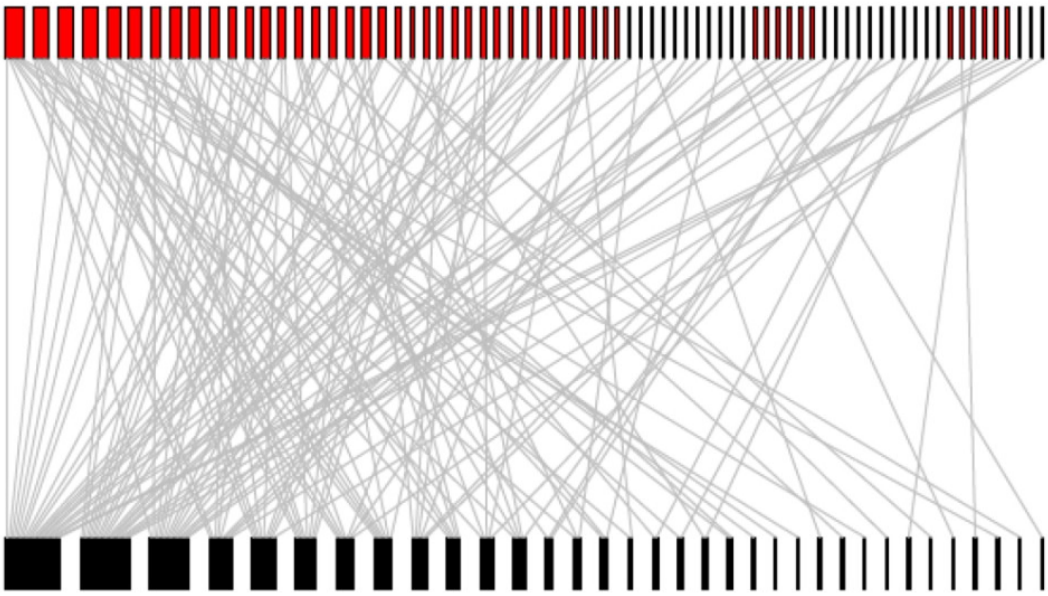

Fig. 1 Co-occurrence of fungal (red box) and bacterial (black box) OTUs from 266 soil samples as calculated from the co-occurrence network analysis. Box size represents the number of strong (rho > 0.5) and significant (p-value < 0.01 after FDR) associations for each OTU.

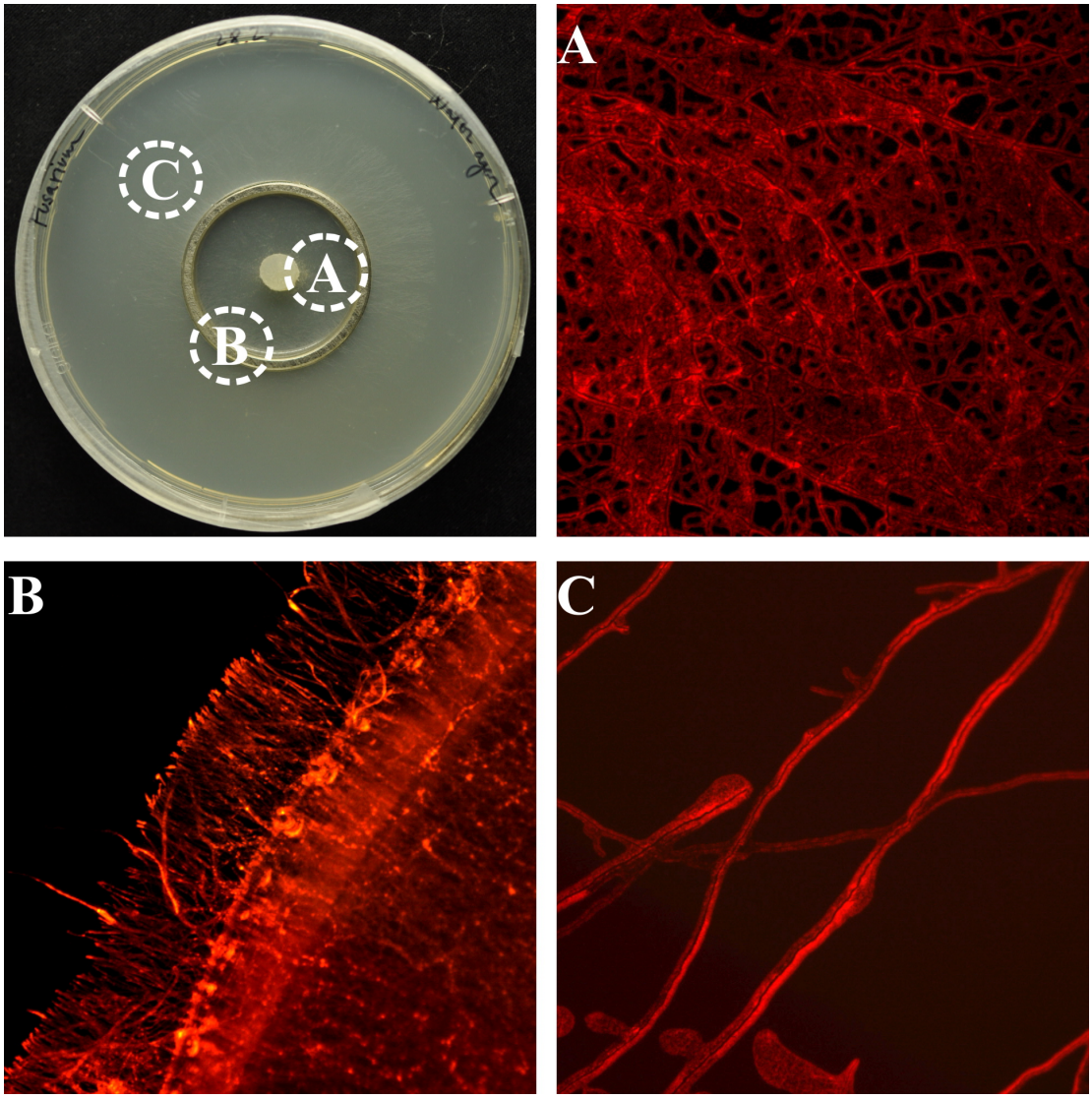

Fig. 2 Monitoring *Burkholderia*-fungi interactions. Plates with sterile iron rings and either water agar, LB or a combination of both were used to monitor the ability to translocate, to disperse and to form biofilms on the hyphae. For microscope observations, *Burkholderia* cells were tagged with gfp or DsRed (on the pictures *B. glathei* LMG14190 tagged with DsRed), co-cultivated with fungi (here *F. solani*) and monitored on daily basis. Microscopy pictures represent different stages of dispersal on *F. solani* hyphae over time. (A) Network of hyphae is densely colonised by bacteria (red) in the inner part of the ring. (B) Crossing of the iron ring by fungal hyphae enables *Burkholderia* cells to cross too when attached to the hyphae. (C) Colonization of the hyphae by transported *Burkholderia* occurs also on the outer part of the iron ring. The same behaviour was visible when water agar was replaced by LB.

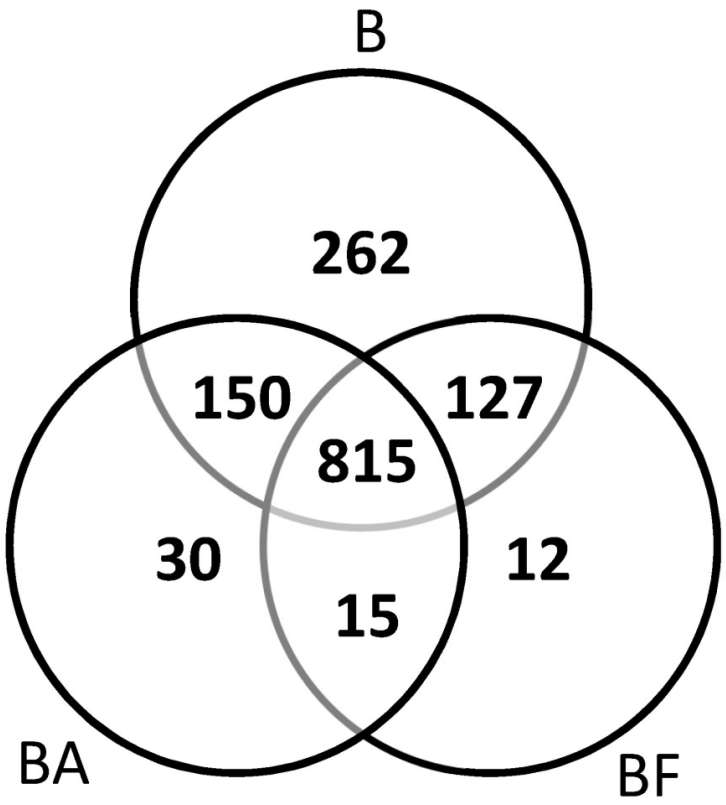

Fig. 3 Venn diagram depicting quantified proteins of *B. glathei* LMG14190 in single culture (B) and in co-cultivation with *A. alternata* (BA) and *F. solani* (BF), respectively.

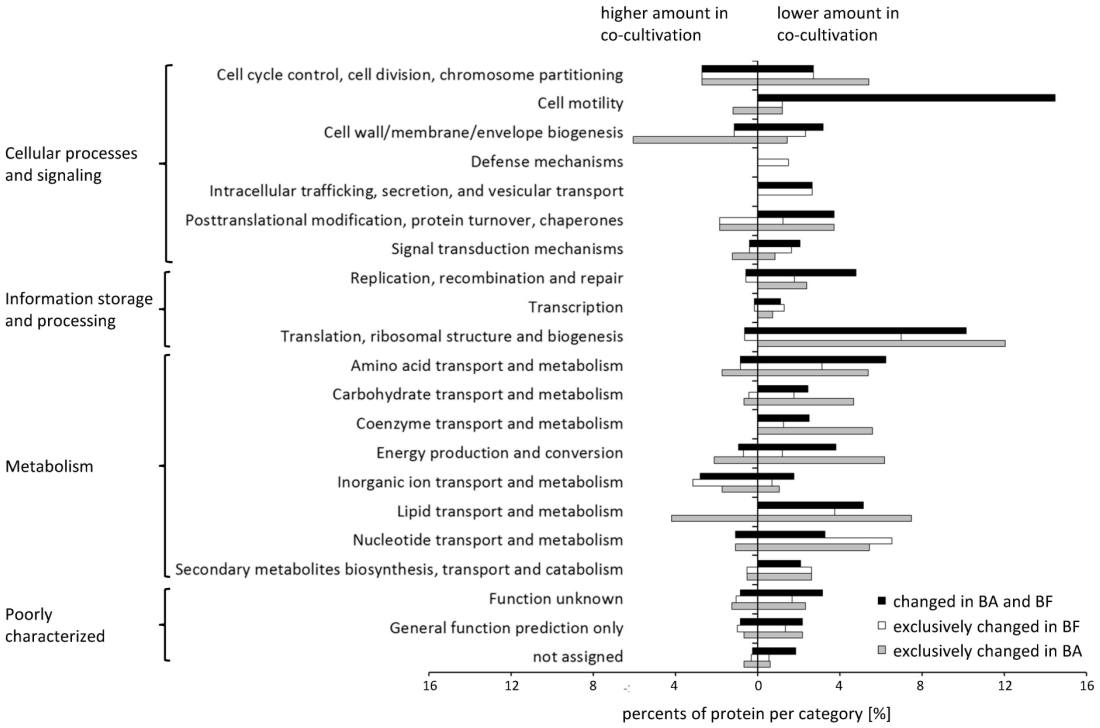

Fig. 4 Impact of co-cultivation on the proteome composition of *B. glathei* LMG14190. The percentage of proteins in relation to the theoretical proteome with at least 2-fold change in co-cultivation with either fungus compared to the control (*B. glathei* alone) is depicted, BA, co-cultivation of *B. glathei* with *A. alternata*, BF, co-cultivation of *B. glathei* with *F. solani*. Black bars indicate proteins with changed amount in both co-cultivations, white bars indicate proteins with changed amounts in the BF co-cultivation, grey bars indicate proteins with changed amounts in the BA co-cultivation. Functional annotation is based on cluster of orthologous groups (COG).

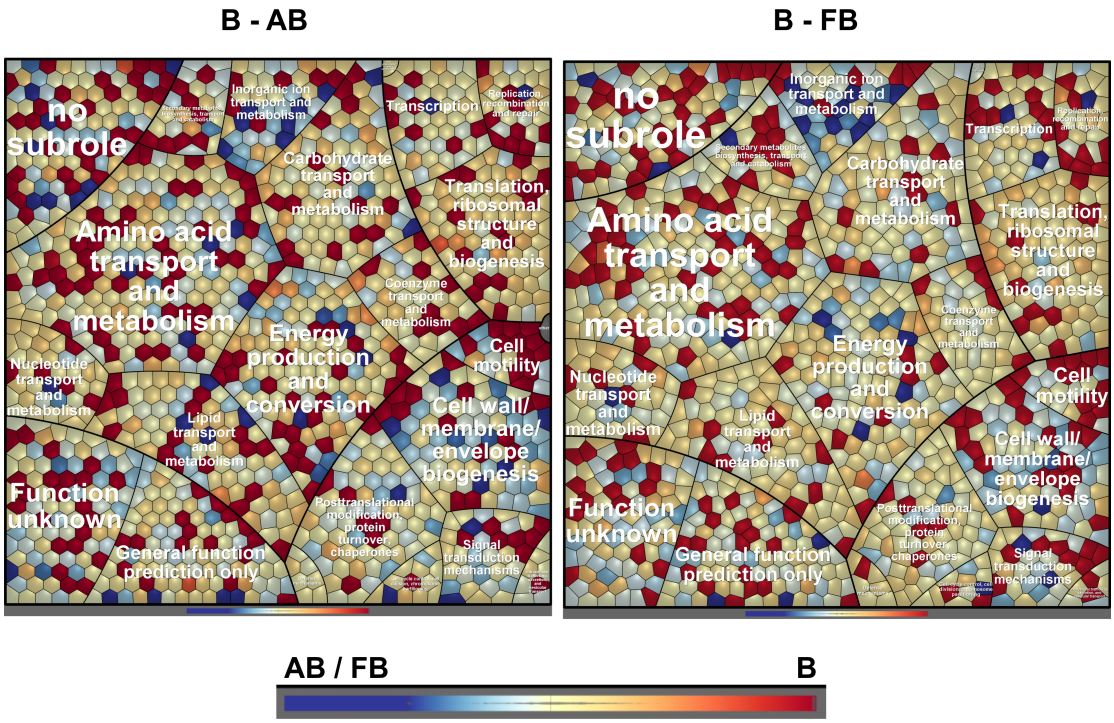

Fig. 5 Voronoi treemaps visualizing changes in the proteome composition of *B. glathei* LMG14190 during fungal co-cultivation. Expression ratios of *B. glathei* LMG14190 proteins in single cultures (B) compared to co-cultivation with *A. alternata* (AB) or *F. solani* (FB). Functional classification of proteins was carried out by Prophane 2.0 and is based on cluster of orthologous groups (COG). Red colour indicates higher expression of the respective protein in single cultivation, blue colour indicates higher expression in co-cultivations. Each cell represents a single protein, functional classes are separated by thicker black lines.

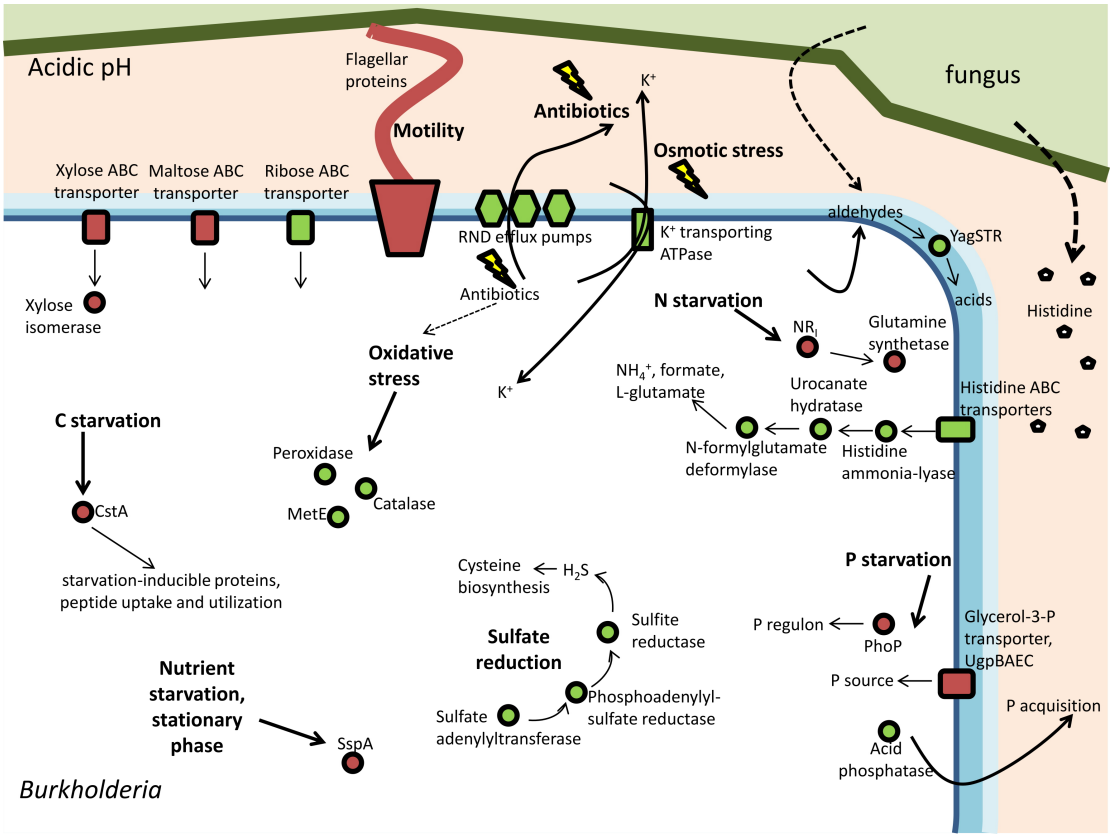

Fig. 6 Schematic overview of the most important changes in *B. glathei* LMG14190 proteome during co-cultivation with *A. alternata* and *F. solani*. The overview includes proteins involved in major pathways that have been detected in our analysis and proteins whose amounts were either decreased (red) or increased (green) in both co-cultivations. Dashed lines represent the effects of hypothetical stress factors (lightning symbols) or substrates that could be produced by fungi. All flagellar proteins were absent in the co-cultivation setups, thus the whole flagella apparatus is marked in red.

Supplementary legends and figures

(The supplementary information can be found in the CD provided)

Supplementary table S1. Co-occurrence network analysis. List of fungal OTUs that co-occurred with the genus *Burkholderia*. Letters d, k, p, c, o, f, g, s correspond to the phylogenetic nomenclature where d – domain, k – kingdom, p – phylum, c – class, o – order, f – family, g – genus and s – species. Sequences are assigned to phylum (p) or even up to species (s) level, depending on the database.

Supplementary tables S2 and S3
S2) Table listing all identified proteins and indicating whether the protein was found in the single and/or co-cultures.
S3) Table listing all quantified proteins together with the NSAF-values, fold-change in expression rates and p-values.

Part III: Role of oxalotrophy in *Burkholderia* – plant interactions

Aims of the project

Oxalate is a common compound in soils as it is produced by a number of plant and fungal species (Libert and Franceschi 1987). It is often accumulated in plant tissues or released by root systems as calcium, iron or magnesium oxalate (Sahin 2003), where it has various functions; calcium (Ca) regulation, plant protection, detoxification and C source for the soil microbiota. In soils where soluble Ca is very abundant, plants can regulate Ca concentration through precipitation of Ca oxalate (CaOx) crystals. These are accumulated mainly in mature plant organs and with time, they can build up to very high levels (85% dry weight of some plants). The protection role is based primarily on temporal, spatial and morphological parameters of CaOx crystal formation. Crystals can have a passive mechanical role (e.g. spikes) or an active biological role (e.g. toxic compound with effect on Ca metabolism) in defense against insects and herbivores (Yoshihara *et al*. 1980, Frutos *et al*. 1998, Tillman-Sutela and Kauppi 1999). In acidic soils, where heavy metal toxicity is a major problem, plants have developed detoxification systems that use organic acids such as malate, citrate and oxalate internally or externally (Ma *et al*. 1998). Most of the oxalate used in these described functions finally ends in the soil environment where, considering the high production by plants and fungi, it should be found in large amounts. However, oxalate is rarely found stored in minerals in the soil, as oxalotrophic microbial communities rapidly consume it as C or energy source (Sahin 2003). Bacteria able to utilize oxalate are phylogenetically distinct and belonging to several genera, such as *Pseudomonas*, *Oxalobacter*, *Ralstonia*, *Starkeya*, *Acetobacter*, *Gluconobacter*, *Xanthobacter*, etc. (Sahin 2003).

The ability to use oxalate as sole C source has been shown also for *Burkholderia* sp. isolated from the rhizosphere of white lupin (Weisskopf *et al*. 2011). Interestingly, the trait was only present in non-pathogenic, environmental *Burkholderia* species and lacking in pathogenic group of BCC and in other plant pathogenic *Burkholderia*. This result, together with the finding that *Burkholderia* species were highly enriched in this system, led us to speculate that oxalate might play a role in *Burkholderia* – plant interactions.

To examine the distribution of the capacity to utilize oxalate a large diversity of *Burkholderia* strains were used in *in vitro* experiments, where oxalate was supplied as sole C source. The role of oxalate degradation in the interaction between *Burkholderia* and plants was investigated by comparing the root and seed colonization of lupin and maize by a *B. phytofirmans* mutant strain impaired in oxalotrophy with that of the corresponding wild-type strain.

frontiers in MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
published: 09 January 2014
doi: 10.3389/fmicb.2013.00421

Oxalotrophy, a widespread trait of plant-associated *Burkholderia* species, is involved in successful root colonization of lupin and maize by *Burkholderia phytofirmans*

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Plant roots and shoots harbor complex bacterial communities. Early seed and plantlet colonization plays a key role in determining which bacterial populations will successfully invade plant tissues, yet the mechanisms enabling plants to select for beneficial rather than harmful populations are largely unknown. In this study, we demonstrate a role of oxalate as a determinant in this selection process, using members of the genus *Burkholderia* as model organisms. Oxalotrophy, i.e., the ability to use oxalate as a carbon source, was found to be a property strictly associated with plant-beneficial species of the *Burkholderia* genus, while plant pathogenic (*B. glumae, B. plantarii*) or human opportunistic pathogens (*Burkholderia cepacia* complex strains) were unable to degrade oxalate. We further show that oxalotrophy is required for successful plant colonization by the broad host endophyte *Burkholderia phytofirmans* PsJN: an engineered Δ*oxc* mutant, which lost the ability to grow on oxalate, was significantly impaired in early colonization of both lupin and maize compared with the wild-type. This work suggests that in addition to the role of oxalate in heavy metal tolerance of plants and in virulence of phytopathogenic fungi, it is also involved in specifically recruiting plant-beneficial members from complex bacterial communities.

Keywords: oxalate, root colonization, *Burkholderia*, PGPR, oxalate decarboxylase

INTRODUCTION

In the rhizosphere, most bacteria rely on root exudates as a source of carbon and energy. Exudates are of highly diverse chemical nature, from small carboxylates to complex phenolic compounds, and their secretion depends mostly on plant species and growth conditions. In nutrient-limited as well as in heavy-metal contaminated soils, exudation of organic acids is increased. This differential exudation of specific compounds has been shown to influence bacterial community structure (Weisskopf et al., 2005, 2008; Badri et al., 2009; Doornbos et al., 2012; Chaparro et al., 2013). Carboxylates such as citrate and malate are a major source of carbon for rhizosphere bacteria, and malate has even been postulated to act as a signal to recruit beneficial microorganisms (Rudrappa et al., 2008). In contrast, using oxalate as carbon source, a phenotype referred to as "oxalotrophy," is a rare trait of bacteria, although it occurs across a wide range of phylogenetically distant groups (Sahin, 2003; Khammar et al., 2009). In addition to citrate and malate, which are common components of root exudates, oxalate has also been shown to be a major root exudate of soil-grown plants (Dessureault-Rompre et al., 2007). However, neither the function of oxalate in recruiting specific microbes nor the relevance of oxalotrophy for bacterial rhizosphere competence has so far been investigated.

Members of the *Burkholderia* genus are frequently retrieved in plant microbiome surveys and seem to play a substantial role in direct plant growth promotion or in protection against soil-borne fungi (Mendes et al., 2007; Opelt et al., 2007; Compant et al., 2008; Li et al., 2008; Hardoim et al., 2011; Ikeda et al., 2013). Yet, beside plant beneficial members of the genus (e.g., *B. phytofirmans, B. phymatum*), others represent a threat to human health, such as the opportunistic pathogens of the *Burkholderia cepacia* complex (Mahenthiralingam et al., 2005). In an effort to characterize the bacterial communities living in and on the roots of white lupin, we have recently shown by both culture-independent and culture-dependent approaches that *Burkholderia* species are predominant members of the bacterial community inhabiting the cluster roots (Weisskopf et al., 2011). In addition to their ability to grow on citrate or malate, almost all isolated *Burkholderia* strains were able to use plant-secreted oxalate as a carbon source: 98% of the *Burkholderia* strains were oxalotrophic, compared with only 2% of the non *Burkholderia* strains isolated from the same environment. Moreover, *Burkholderia* sequences and strains almost exclusively belonged to the plant beneficial species and not to the opportunistic pathogenic ones (Weisskopf et al., 2011). These results led us to hypothesize that the capacity to utilize plant-exuded oxalate might explain why the roots of white lupin are strongly enriched for *Burkholderia* species. To test this hypothesis, we determined the capacity to utilize oxalate among a wide range of *Burkholderia* strains that belong either to plant beneficial or to opportunistic pathogenic species. In addition, we mutated

the oxalotrophy pathway in the plant beneficial endophytic *B. phytofirmans* and monitored seed and root colonization of the mutant and the wild-type strains in white lupin and in maize.

MATERIALS AND METHODS

STRAINS, PLASMIDS AND CULTURE MEDIA

Strains and plasmids used in this study are listed in **Table S1**. For long-term storage, bacterial strains were kept at −80°C in 50% glycerol. Chemicals were purchased from Sigma Aldrich if not specified otherwise. Bacteria were routinely grown on Luria-Bertani (LB) medium (20 g LB powder (Difco) per liter) and 18 g agar, *Pseudomonas* Isolation Agar (PIA) medium (45 g *Pseudomonas* Isolation Agar (Difco), 5 g additional agar, 20 ml glycerol per liter), or Mueller-Hinton agar (21 g Mueller Hinton Broth (Difco) and 15 g agar per liter). For oxalate degradation assay, AB minimal medium was used with (per liter) 2 g $(NH_4)_2SO4$, 6 g Na_2HPO_4, 6 g Na_2HPO_4, 3 g NaCl, 2 mM $MgCl_2 \times 6\ H_2O$, 100 µM $CaCl_2 \times 6\ H_2O$, 3 µM $FeCl_3 \times H_2O$ and 40 µl oligoelement solution (10 mg $ZnSO_4 \times 7\ H_2O$, 13 mg $MnCl_2 \times 4\ H_2O$, 3 mg $Na_2MoO_4 \times 2\ H_2O$, 30 mg H_3Bo_3, 20 mg $CoCl_2 \times 6\ H_2O$, 1 mg $CuCl_2 \times H_2O$, 2 mg $NiCl_2 \times 6\ H_2O$). pH was adjusted to 7. This medium, supplemented with 18 g agar per liter, was used as the first layer of the oxalate degradation medium. A second layer, which contained 7 g calcium oxalate $\times\ H_2O$ and 12 g agar per liter was freshly stirred and added on the first layer. MS medium contained 2.2 g Murashige and Skoog medium (Sigma-Aldrich) and 5 g agar per liter. pH was adjusted to 5.7 prior to autoclaving.

OXALATE DEGRADATION ASSAY

Strains were grown overnight in 5 ml of AB minimal medium with 5 g l^{-1} glucose as carbon source. 2 ml of the overnight culture were centrifuged at 4000 rpm for 5 min and the pellet was washed twice and resuspended in 1 ml 0.9% NaCl solution. OD_{600} was measured and all samples were diluted with 0.9% NaCl to OD_{600} of 0.2. 50 µl of diluted cell suspension were pipetted onto the double layer oxalate-medium and incubated for at least 2 days at 30°C. The formation of a transparent halo revealed the ability to degrade oxalate.

CONSTRUCTION OF A MUTANT IMPAIRED IN OXALATE DEGRADATION AND FLUORESCENT TAGGING

In *B. phytofirmans* PsJN, the oxalate degradation cluster is located on chromosome 2 and consisted of three genes encoding (i) the oxalate/formate antiporter (Bphyt_6739), (ii) the oxalate decarboxylase (*oxc*, Bphyt_6740), and the formyl-CoA transferase (*frc*, Bphyt_6741) (Weilharter et al., 2011). Unlike the antiporter and the formyl-CoA transferase, the oxalate decarboxylase was present as single copy in the genome, and was thus chosen as a target for mutagenesis. A 1650 bp region spanning Bphyt_6740 (*oxc*) was amplified using XhoI and BglII restriction site-containing primers 5′-GCGCCTCGAGCTGAACGACATCAAAACCAT-3′ and 5′-GCGCAGATCTGATTACTTTTTCATTGCCGC-3′, which were designed using the CLC workbench software and purchased from Microsynth, Balgach, Switzerland. The PCR reaction was performed as follows: 1 cycle of 2 min at 95°C followed by 30 cycles of 30 s at 94°C, 30 s at 48°C, and 100 s at 72°C, and a final extension at 72°C for 5 min. The resulting amplicon was purified using Qiagen PCR purification kit, digested with BglII and XhoI and ligated overnight at room temperature with the vector pSHAFT2 (4552 bp) previously digested with the same enzymes. The ligation product was transformed into *E. coli* CC118λpir cells followed by selection for chloramphenicol resistant clones on LB plates. The resulting plasmid (pSHAFT2 carrying *oxc*) was then isolated and digested with NcoI, a restriction site located in the middle of *oxc*, dephosphorylated and purified. In parallel a trimethoprim resistance cassette was amplified by PCR using NcoI containing primers 5′-GCGCCCATGGCAGTTGACATAAGCCTGTTC-3′ and 5′-GCGCCCATGGTTAGGCCACACGTTCAAGTG-3′, which were designed using the CLC workbench software and purchased from Microsynth, Balgach, Switzerland. The PCR reaction was performed by 1 cycle of 2 min at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 50°C, and 100 s at 72°C, and a final extension at 72°C for 5 min. The resulting amplicon was digested with NcoI and purified. Ligation was performed overnight and the ligation product was transformed into CC118λpir cells. Clones were selected on Mueller-Hinton plates supplemented with trimethoprim and correct insertion in the isolated plasmids was verified by restriction with NcoI or XhoI and BglII. This strain carrying the interrupted *oxc* gene was used as donor strain for triparental mating with *E. coli* MM294 strain as a helper and *B. phytofirmans* PsJN as a recipient. 2 ml of overnight culture (5 ml LB medium with appropriate antibiotic) was centrifuged and washed twice in 0.9% NaCl solution. Then the cells were resuspended in 0.5 ml LB media. 100 µl of the helper culture; 100 µl of the donor strain culture were mixed and kept at room temperature (RT) for 20 min and then 100 µl of recipient strain were added. Afterwards 150 µl of the mixed culture were pipetted in drops of about 50 µl on a LB plate and incubated for 6 h at 30°C. Then the cells were harvested, resuspended in 1.5 ml 0.9% NaCl solution, diluted and spread on PIA plates supplemented with trimethoprim. Loss of chloramphenicol resistance was used to select clones where double crossing-over recombination had occurred (see **Figure S1** for a diagram of the cloning procedure). Fluorescent tagging of *B. phytofirmans* wild-type and Δ*oxc* mutant was carried out by triparental mating as described above. The donor strains were *E. coli* carrying either the plasmid pBBR1MCS-2-gfpmut3 (GFP, kanamycin resistance) or the plasmid pIN62 carrying the dsRED encoding gene and a chloramphenicol resistance cassette (see **Table S1**). Transformants were selected on PIA plates with kanamycin or chloramphenicol.

PLANT COLONIZATION EXPERIMENTS

Early colonization

Two plant species were used as models for the colonization assays of *B. phytofirmans* wild-type and Δ*oxc*-mutant: white lupin (*Lupinus albus* L., cv. Amiga) and maize (*Zea mays* subsp. *mays*, cv. Birko). Seeds were sterilized by vigorous shaking (200 rpm) in 2.5% NaHClO solution 0.2‰ (v:v) Triton X for 5 min, followed by rinsing twice in sterile water and drying under the sterile bench. Seeds were bacterized with *B. phytofirmans* strains using the following procedure: dsRED- or GFP-tagged derivatives of the wild-type strain and the Δ*oxc* mutant, were grown

overnight in LB broth. The dsRED-tagged strains were used for single inoculation experiments due to the higher signal intensity compared to the GFP-tagged cells. Cells were harvested by centrifuging for 5 min at 6000 rpm, washed twice in NaCl 0.9% and resuspended in 20 ml NaCl solution to adjust the OD_{600} to 0.25 (corresponding to approximately 10^7 cells/ml). For mixed inoculations (GFP-tagged wild-type (wt): dsRED-tagged Δ*oxc*, dsRED-tagged wt: GPF-tagged Δ*oxc*), the two strains were mixed after cell washing in a 1:1 ratio (OD_{600} of 0.125 for each strain). 20 surface-sterilized seeds of maize or 20 seeds of lupin were dipped in 10 ml of the respective bacterial suspension and incubated in a Falcon tube for 1 h at room temperature. Control seeds were incubated in NaCl solution. Thereafter, bacterized seeds were washed in NaCl to remove non-attached cells and sterilely transferred to Petri dishes with 1/2 MS medium. Plates were incubated for 3 days at room temperature in the dark to allow seed germination. After 3 days, selected germinated seeds were examined with a Leica M165FC fluorescent microscope for colonization pattern while other seeds from the same batch were used for colony forming unit (CFU) determination. For the latter, germinated seeds were placed in a 15 ml Falcon tube filled with 10 ml NaCl 0.9% and gently detached by 15 min incubation in a sonication water bath (Memmert WB 14, Germany). Thereafter, the cell suspensions were serially diluted and plated on PIA medium. Colonies were counted after four day incubation at 30°C. To verify statistical significance student's *t*-test was performed. For dual inoculation, colonies were counted under the binocular (to verify green fluorescence, which was not visible by eye on the plate unlike the red color originating from dsRED-tagging). After 7 days of incubation in the Petri dish that contained 1/2 MS medium, new seedlings were harvested and examined for early colonization pattern using a NightOWL LB 983 NC100 (Berthold technologies, Germany).

Persistence in planta

Seeds of maize and lupin were bacterized using the procedure described above. After 3 days of germination, four seeds for each treatment were transferred to 50 ml Falcon tubes filled with vermiculite (one seed per tube). Plants were transferred to a greenhouse with natural light, approximately 25°C and 70% humidity. 7 days later, a second inoculation step was carried out on these vermiculite microcosms by adding 4 ml of a cell suspension adjusted to an OD_{600} of 0.25 to each Falcon (NaCl for the control microcosms). Plants were fertilized once a week with MIOPLANT fertilizer (Migros, Switzerland) using half the concentration recommended by the manufacturer and watered twice a week. They were harvested after 28 days. To determine CFUs, roots were gently ground in NaCl 0.9% and ground tissues serially diluted and plated on PIA plates (see above, Early colonization).

OXALATE MEASUREMENTS IN PLANT TISSUES

Oxalate measurements in lupin and maize root tissues were performed after 3 and 28 days with an enzymatic kit from LIBIOS (France). Prior to analysis, washed roots were weighted and ground in liquid N_2. The resulting powder was extracted in twice its weight of water for 30 min under continuous shaking. Thereafter, the extract was centrifuged at 13,000 rpm for 5 min and 10 µl of the supernatant was used for oxalate quantification according to the manufacturer's protocol.

CONSTRUCTION OF PHYLOGENETIC TREE OF *BURKHOLDERIA* SPECIES

Forty one *Burkholderia* 16S rRNA gene sequences were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/). 1130 bp long sequences were aligned using ClustalW (Thompson et al., 1994) in MEGA5.05 software (Tamura et al., 2011). Phylogenetic trees were obtained by applying the Neighbor-Joining (NJ) method in the MEGA 5.05 software. The tree topology was inferred with a Kimura 2-parameter correction model (Kimura, 1980) and with 1000 bootstrap replications. 16S rRNA gene sequence of *Ralstonia solanacearum* LMG 2299 was used as an outgroup.

RESULTS

OXALOTROPHY IS WIDESPREAD IN PLANT-ASSOCIATED *BURKHOLDERIA* SPECIES BUT ABSENT FROM OPPORTUNISTIC PATHOGENIC SPECIES

Fifty eight strains, which belong to 41 different species were tested for their ability to utilize oxalate as a sole carbon source. None of the strains from the *Burkholderia cepacia* complex species could grow on oxalate (**Table 1**, **Figure 1**). Likewise, all plant pathogenic *Burkholderia*, including strains of *B. glumae*, *B. plantarii*, and *B. gladioli* were unable to do so. In contrast, all *Burkholderia* strains that belonged to the "plant beneficial cluster" (Suarez-Moreno et al., 2012) were oxalotrophic, with the exception of *B. phenazinium*, which could not grow on oxalate (**Table 1**) and from which the *frc* gene [formyl-CoA transferase, catalyzing the first step of oxalate catabolism (Khammar et al., 2009)] could not be amplified (data not shown). The ability or inability to degrade oxalate was conserved within the same species, as shown for diverse examples (**Table 1**). The almost universal trait of plant-associated *Burkholderia* to utilize oxalate and the incapacity of all tested plant or human opportunistic pathogens to do so led us to hypothesize that oxalotrophy might be involved in the establishment of mutualistic interactions between bacteria and plants.

OXALOTROPHY IS INVOLVED IN SUCCESSFUL PLANT COLONIZATION BY *B. PHYTOFIRMANS*

To evaluate the role of oxalotrophy in plant colonization, the oxalate decarboxylase gene *oxc* was inactivated in the broad-host endophytic bacterium *B. phytofirmans* PsJN (Sessitsch et al., 2005). The *oxc* gene is the second gene in a putative oxalate catabolism gene cluster, which contains the putative oxalate/formate antiporter Bphyt_6739, *oxc*, and the formyl-coA transferase gene *frc* (Bphyt_6741) (**Figure S1**). As expected, oxalotrophy was abolished in the mutant strain (**Figure 2**). The wild-type and the Δ*oxc* mutant were marked with either GFP or dsRED to allow monitoring of their plant colonization abilities (see Materials and Methods for details). The marked strains exhibited the same growth behavior in LB medium in single as well as in mixed inoculation experiments, indicating that the marker genes (GFP, dsRED) did not affect the results (**Figure S2**).

Sterilized seeds of lupin and maize were inoculated with (i) the wild-type, (ii) the Δ*oxc* mutant, and (iii) both strains

Table 1 | Oxalate degradation ability (OX) in various species of the *Burkholderia* genus.

Species	Strain	OX
Plant beneficial environ. *Burkhoderia* sp.		
B. caledonica	LMG19076	+
B. caribensis	LMG18531	+
B. caryophylli	LMG2155	+
B. bryophila	LMG23646	+
B. fungorum	LMG16225	+
B. graminis	LMG18924	+
B. hospita	LMG20598	+
	Isolate NS11	+
	Isolate NS7	+
B. kururiensis	LMG19447	+
B. phenoliruptrix	LMG22037	+
B. phymatum	LMG21445	+
B. phytofirmans	LMG22487	+
B. sacchari	LMG19450	+
B. terricola	FN313521	+
	LMG20594	+
B. tropica	LMG22274	+
B. tuberum	LMG21444	+
B. xenovorans	LMG21463	+
B. phenazinium	LMG2247	–
	Isolate S1	–
	Isolate S7	–
	Isolate S18	–
	Isolate 1S9	–
Unclassified *Burkholderia* sp.		
B. glathei	LMG14190	+
B. sordidicola	LMG22029	+
B. thailandensis	LMG20219	–
B. andropogonis	LMG2129	–
Species	**Strain**	**OX**
***Burkholderia cepacia* complex sp.**		
B. ambifaria	LMG17828	–
B. anthina	LMG21821	–
B. arboris	LMG24066	–
B. cenocepacia	R−6274	–
B. cepacia	ATCC25416	–
B. contaminans	LMG23361	–
B. diffusa	LMG24065	–
B. dolosa	LMG18941	–
B. lata	LMG22485	–
B. latens	LMG24064	–
B. metallica	LMG24068	–
B. multivorans	LMG18825	–
B. pyrrocinia	LMG14191	–
	LMG21822	–
	LMG21823	–
B. seminalis	LMG24067	–
B. stabilis	Isolate R6270	–
	LMG14294	–
B. ubonensis	LMG20358	–
B. vietnamiensis	LMG18835	–
Plant pathogenic *Burkholderia* sp.		
B. gladioli	LMG2216	–
	LMG11626	–
	LMG18157	–
B. glumae	LMG2196	–
	ATCC33617	–
	AU6208	–
	ATCC43733	–
B. plantarii	Isolate TT	–
	Isolate VV	–
	LMG9035	–

Oxalate degradation for pure Burkholderia cultures was revealed by a halo surrounding growing colonies when inoculated on a minimal medium with calcium oxalate as a sole carbon source (see Materials and Methods for details).

in equal cell densities (approximately 10^7 cells/ml of inoculation solution). For single inoculation studies, the dsRED-tagged strains were used, as the signal was brighter than in the GFP-tagged strains. For dual inoculations, both combinations were used (GFP-tagged wild-type and dsRED-tagged Δ*oxc*, or dsRED-tagged wild-type and GFP-tagged Δ*oxc*) to avoid any bias due to fluorescent marker genes. When inoculated as single strains, a significant decrease in root colonization capacity was observed in the mutant relative to the wild-type on both lupin and maize (**Figure 3A**). This difference, which was confirmed by microscopic inspection (**Figure 4**), was more pronounced at early stages of colonization than after one month of cultivation, especially for maize. In lupin, about a million cells/g root fresh weight could be detected for the wild-type in all three plants after 28 days, yet the mutant was only detectable in one of three plants and present at a much lower population density (100-fold decreased relative to the wild-type). In maize, the difference was less pronounced after one month of cultivation when compared to the beginning of colonization (just below significance level, $P = 0.055$). This difference might be explained by the fact that lupins produced much more oxalate than maize (30 nmol vs. 6 nmol per g root fresh weight after 3 days and 60 nmol vs. 30 nmol after 28 days). When inoculated together with the wild-type, the colonization defect of the Δ*oxc* mutant was restored (**Figure 3B**), that was confirmed by visual inspection of 7 day-old seedlings (**Figure 5**). While the Δ*oxc* mutant was not able to spread from the seeds to the roots when inoculated as a pure culture (**Figure 5C**), this phenotype was partially rescued in the presence of the wild-type strain (**Figure 5D**).

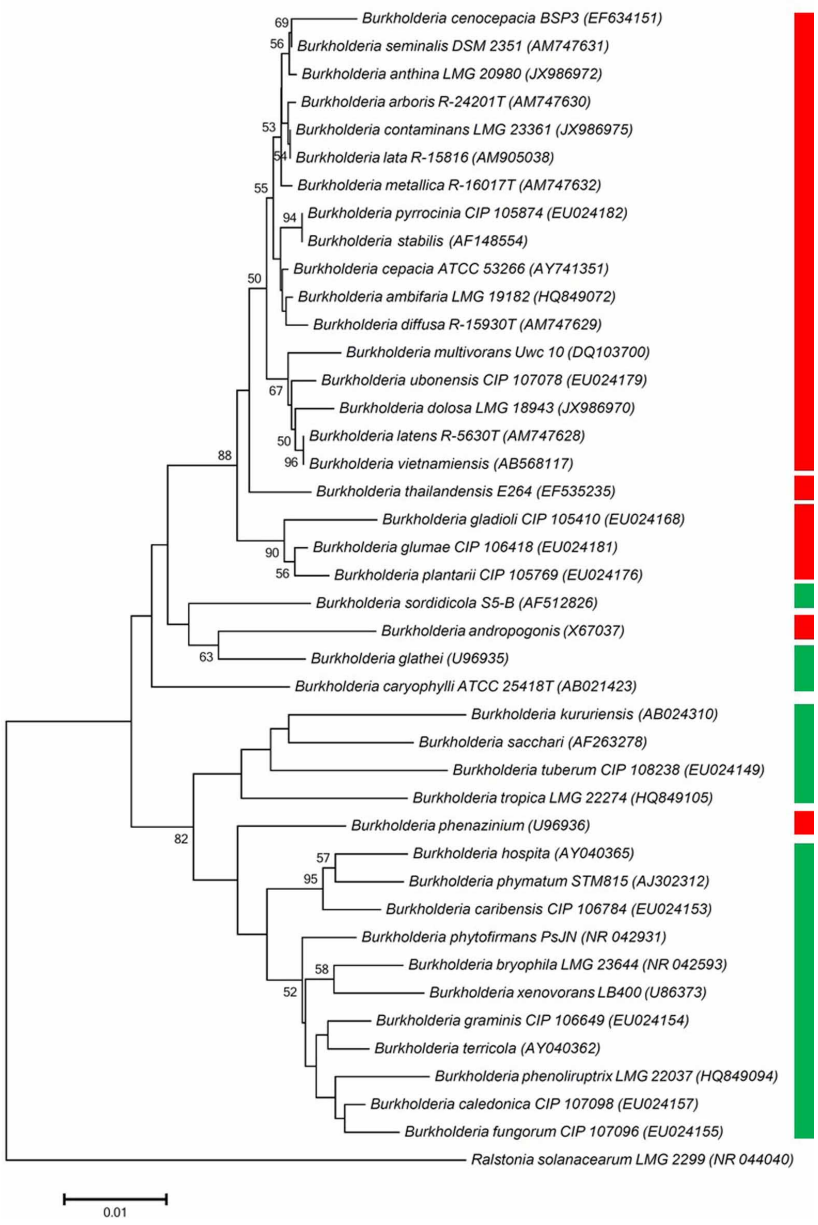

FIGURE 1 | Phylogenetic tree, constructed using one representative 16S rRNA gene sequence per *Burkholderia* species included in the oxalotrophy assay. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Only bootstrap values exceeding 50% are labeled. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Green bar indicates oxalotrophy of the tested strains of a given species, red bar indicates inability to degrade oxalate (see also **Table 1** for detailed results).

DISCUSSION

One major source of oxalate in natural ecosystems is fungal production, e.g., in wood-rotting fungi, where it is involved in lignin degradation or in some phytopathogenic fungi, e.g., *Sclerotinia* or *Botrytis* species, where it acts as virulence factor (Dutton and Evans, 1996; Criscitiello et al., 2013; Heller and Witt-Geiges, 2013). High quantities of oxalate are toxic to animals and humans, due to the formation of calcium- or magnesium oxalate crystals, which can lead to depletion in essential cations or to kidney stone formation (Coe et al., 2010). However, oxalate is also an important metabolite of many plant species, where it is thought to be important for calcium storage and for repelling herbivores (Franceschi and Nakata, 2005). Moreover, oxalate secretion is involved in tolerance to heavy metals including aluminum, as demonstrated e.g., in buckwheat (Klug and Horst, 2010) or in rice (Yang et al., 2000).

When plants grow in situations where nutrients such as phosphate or iron are limited, or when heavy metals are abundant, excretion of organic acids is increased (Meyer et al., 2010). This enhanced secretion of citrate, malate or oxalate enriches the rhizosphere in organic carbon, which can be used by certain microorganisms as a nutritional source. Consequently, those members of the community that possess the metabolic means to catabolize those exudates will be enriched. In a previous study, we observed an overrepresentation of *Burkholderia* species in various development stages of white lupin cluster roots (Weisskopf et al., 2011). This enrichment might be linked to the acidic environment that prevails around mature cluster roots and to the preference of *Burkholderia* species to exist in acidic soils (Stopnisek et al., 2013). Given that most of the *Burkholderia* strains isolated from white lupin were able to utilize oxalate as a carbon source, we asked whether this property is, like acid tolerance, a genus-wide property or is restricted to species that are predominantly associated with plants and/or fungi. By testing strains that belong to 41 different species, we observed that the ability to grow on oxalate as a sole carbon source is restricted to members of the plant-beneficial environmental cluster (Suarez-Moreno et al., 2012) (**Figure 1**) and absent in pathogenic species, including the human pathogen *B. pseudomallei*, plant pathogens such as *B. plantari* or *B. glumae* and opportunistic pathogens, which belong to the Bcc cluster. Interestingly, virulent strains of *B. glumae*, an important pathogen of rice, have been shown to produce oxalate, while non-virulent ones were not oxalogenic (Li et al., 1999), suggesting that oxalate production might be important for virulence, as it is the case with fungal pathogens. Beyond its role as a virulence factor, oxalate has been postulated to be a common good of pathogenic *Burkholderia* species, including *B. glumae, B. pseudomallei and*

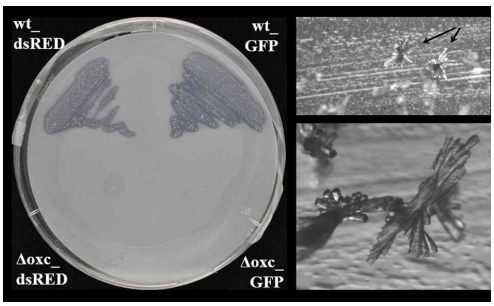

FIGURE 2 | Assessment of oxalotrophy in the dsRed and GFP-tagged wild-type (wt_dsRed respectively wt_GFP) and the accordingly tagged (Δ*oxc*_dsRED respectively Δ*oxc*_GFP) Δ*oxc* mutants of *B. phytofirmans* (left) strain. Both tagged wild-type strains (upper half of the Petri dish) showed a cleared halo around the grown colonies, which indicates degradation of the Ca-oxalate present in the upper layer of the minimal medium. Both tagged Δ*oxc* mutant strains (lower half of the Petri dish) were unable to grow on the minimal medium with oxalate as a sole carbon source. Picture was taken after 10 days of growth at room temperature. In the oxalate degrading strains, characteristic crystal structures (most probably $CaCO_3$) were formed above the agar surface (right, arrows, and zoomed view below).

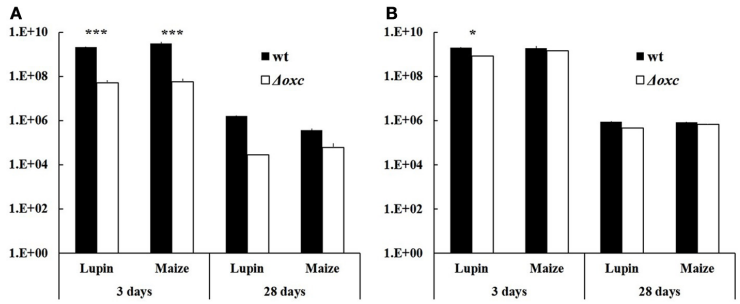

FIGURE 3 | Abundance of *B. phytofirmans* PsJN wild-type and Δ*oxc* mutant after seed germination (3 days) and after one-month microcosm cultivation in vermiculite (28 days). Seeds were either bacterized with one strain (single inoculation, **A**) or with a 1:1 mixture of wt and Δ*oxc* mutant (dual inoculation, **B**). Results are expressed as CFU per seed after 3 days and as CFU per g root fresh weight after 28 days. Average of 2–3 replicates (seeds/plants) are shown, with the exception of one case, where no mutant cells were retrieved in two out of three replicate plants (**A**, lupin, 28 days, Δ*oxc*). Stars indicate significant differences between wild-type and mutant (Student's *t*-test, $n = 2$–4, $^{*}P < 0.05$, $^{***}P < 0.001$).

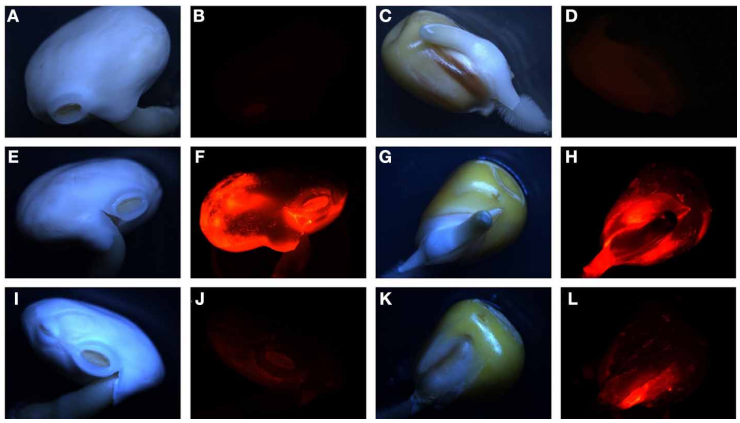

FIGURE 4 | Representative pictures of seed colonization of lupin (A,B,E,F,I,J) and maize (C,D,G,H,K,L) by dsRED-tagged wild-type (E–H) or Δ*oxc* mutant (I–L) after 3 days. A-D: non inoculated seeds. Pictures were taken using a Leica M165FC fluorescent microscope, under normal light **(A,C,E,G,I,K)** or dsRED fluorescent filter **(B,D,F,H,J,L)** with 0.4 s. exposure in all cases.

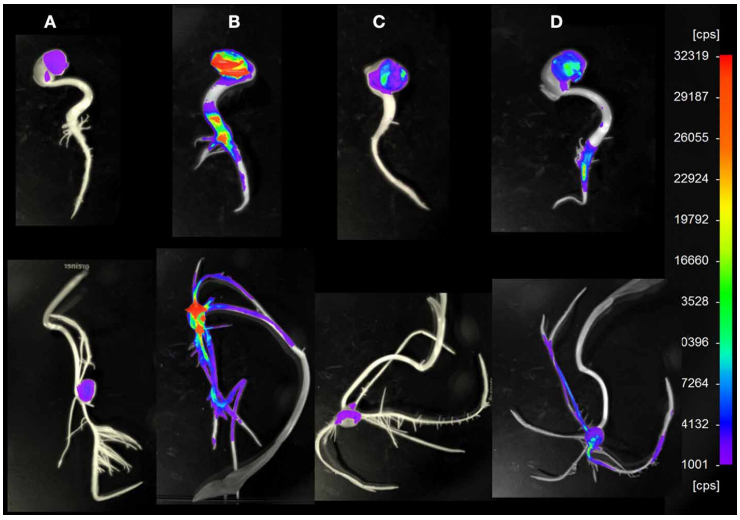

FIGURE 5 | Representative pictures of lupin (upper row) and maize (lower row) 7 day old seedlings colonized by *B. phytofirmans* PsJN wild-type or Δ*oxc* mutant in single or combined inoculation. For imaging, a NightOWL LB 983 NC100 was used, under conditions where dsRED-tagged cells are visible. **(A)**: non inoculated control, **(B)**: inoculated with dsRED-tagged wild-type, **(C)**: inoculated with dsRED-tagged Δ*oxc*, **(D)**: inoculated with GFP-tagged wild-type and dsRED-tagged Δ*oxc* mutant. cps: counts per second.

B. thailandensis (Goo et al., 2012). Oxalate production in these species is controlled by quorum-sensing and was shown to neutralize the alkalinization of the medium caused by the emission of NH_3 in the late stationary phase, thereby ensuring that the pH remains at a physiological level (Goo et al., 2012).

Oxalate degradation by plant-beneficial *Burkholderia* might be considered a plant-protecting feature, as lowering the oxalate levels on plant surfaces might alleviate the infection potential of oxalate-producing phytopathogenic fungi or bacteria. This was shown in the case of *Cupriavidus campinensis*, which could significantly reduce disease symptoms caused by the oxalogenic fungi *Botrytis cinerea* or *Sclerotinia sclerotiorum* on *Arabidopsis*, grapevine and tomato plants, while a mutant strain impaired in oxalate degradation showed only reduced protecting potential

(Schoonbeek et al., 2007). In order to investigate whether oxalate degradation might provide an advantage in plant colonization, a mutant, in which oxalotrophy is abolished, was generated (**Figure 2**). The broad-host endophyte *B. phytofirmans* PsJN (Sessitsch et al., 2005) served as a model organism in this study. The colonization behavior of the wild-type and the mutant on plants with moderate (white lupin) or low (maize) oxalate secretion was compared. When inoculated alone, the mutant suffered a drastic disadvantage both in early colonization steps (3 days) and in persistence on the plants (**Figures 3A**, **4**, **5**). Similar differences between the wild-type and the mutant were observed for lupin and maize at the early stage of colonization; however, after one month of cultivation the effects were much more dramatic on lupins, where only in one out of three plants mutant cells could be recovered, than on maize, for which the difference between wild-type and mutant was not significant. Surprisingly, when the mutant and the wild-type were inoculated in a 1:1 ratio, the mutant recovered most of its lost capacity to colonize the plants (**Figures 3B**, **5**). This suggests that oxalate might act as a toxic compound for the strains that cannot degrade it. The presence of the wild-type would then alleviate this toxic effect by lowering the levels of free oxalate through oxalotrophy. When grown in glucose-supplemented minimal medium, the mutant's growth was only very marginally reduced upon addition of oxalate, which indicates that oxalate is not toxic under laboratory conditions. However, this does not exclude a putative toxicity of oxalate in the seed or plant environment. Moreover, the better colonization performance of the mutant when co-inoculated with the wild-type might also be explained by the utilization of degradation products resulting from oxalate catabolism of the wild-type.

Roots are the entry point for most endophytic bacteria, which then can spread to above-ground plant tissues. Understanding how plants select for beneficial root and shoot inhabitants and/or against plant pathogenic species is obviously very important for plant health. This work sheds light on the so far overlooked role of oxalotrophy in root colonization, which in the case of *Burkholderia* species selects for plant beneficial bacteria over colonization by plant and even animal pathogens.

AUTHOR CONTRIBUTIONS

Laure Weisskopf designed the research, Thomas Kost, Kirsty Agnoli and Laure Weisskopf performed experiments, Thomas Kost, Nejc Stopnisek, Kirsty Agnoli and Laure Weisskopf analyzed the data, Laure Weisskopf wrote the MS with help from Kirsty Agnoli, Nejc Stopnisek, Thomas Kost, and Leo Eberl.

ACKNOWLEDGMENTS

The authors wish to thank P. Junier, G. Cailleau and G. Martin for fruitful discussions and A. Vergunst for providing plasmid pIN62. We are thankful to Stefanie Heller for help with microscopic observations. Financial support of the Swiss National Science Foundation (grant 31003A-130089 to Laure Weisskopf) is gratefully acknowledged.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Journal/10.3389/fmicb.2013.00421/abstract

Table S1 | Strains and plasmids used in this study.

Figure S1 | Structure of the gene cluster involved in oxalate degradation and construction of a Δ*oxc* mutant in *B. phytofirmans* PsJN.

Figure S2 | Growth curves and *in vitro* competition experiment of *B. phytofirmans* PsJN wt and Δ*oxc* mutant.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 September 2013; accepted: 23 December 2013; published online: 09 January 2014.

*Citation: Kost T, Stopnisek N, Agnoli K, Eberl L and Weisskopf L (2014) Oxalotrophy, a widespread trait of plant-associated Burkholderia species, is involved in successful root colonization of lupin and maize by Burkholderia phytofirmans. Front. Microbiol. **4**:421. doi: 10.3389/fmicb.2013.00421*

This article was submitted to Plant-Microbe Interaction, a section of the journal Frontiers in Microbiology.

Table S1 Strains and plasmids used in this study. References are listed for the strains without ATCC or LMG number. CF: cystic fibrosis; wt: wild-type; Δ*oxc*: mutant strain with interrupted oxalate decarboxylase gene; GFP: green fluorescent protein; dsRED: red fluorescent protein.

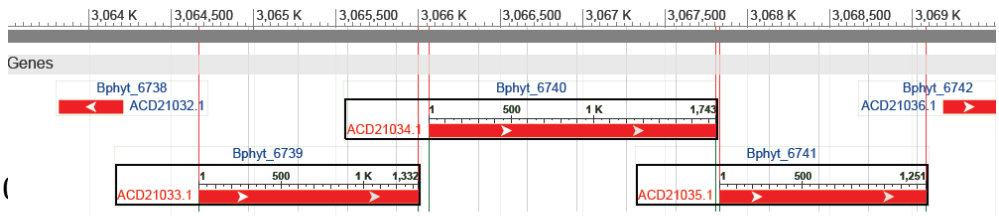
Species	**Strain number**	**Strain origin**	**Reference**
B. ambifaria	LMG17828	roots	
B. andropogonis	LMG2129	*Sorghum bicolor*	
B. anthina	LMG21821	CF patient	
B. arboris	LMG24066	soil	
B. bryophila	LMG23646	moss	
B. caledonica	LMG19076	soil rhizosphere	
B. caribensis	LMG18531	soil	
B. caryophylli	LMG2155	*Dianthus caryophyllus*	
B. cenocepacia	R-6274	CF patient	Gotschlich *et al*. 2001
B. cepacia	ATCC25416	*Allium cepa*	
B. contaminans	LMG23361	sheep milk	
B. diffusa	LMG24065	CF patient	
B. dolosa	LMG18941	CF patient	
B. fungorum	LMG16225	*Phanerochaete chrysosporium*	
B. gladioli	LMG2216	*Gladiolus* sp.	
B. gladioli	LMG18157	CF patient	
B. gladioli	LMG11626	poisoned bongkrek	
B. glathei	LMG14190	soil	
B. glumae	LMG2196	*Oryza sativa*	
B. glumae	AU6208	clinical isolate	
B. glumae	ATCC33617	*Oryza sativa*	
B. graminis	LMG18924	roots	
B. hospita	NS7	soil	this study
B. hospita	NS11	soil	this study
B. hospita	LMG20598	soil	
B. kururiensis	LMG19447	water	
B. lata	LMG22485	soil	
B. latens	LMG24064	CF patient	
B. metallica	LMG24068	clinical isolate	
B. multivorans	LMG18825	CF patient	
B. phenazinium	S1	moss	Opelt and Berg 2004
B. phenazinium	S7	moss	Opelt and Berg 2004
B. phenazinium	S18	moss	Opelt and Berg 2004
B. phenazinium	1S9	moss	Opelt and Berg 2004
B. phenazinium	LMG2247	soil	
B. phenoliruptrix	LMG22037	chemostat	
B. phymatum	LMG21445	root nodules	
B. phytofirmans	LMG22487 (PsJN)	onion roots	
B. phytofirmans Δ*oxc* (pin62)	LMG22487	dsRED-tagged wild-type PsJN	this study
B. phytofirmans (pBBR1MCS-2-gfpmut3-1)	LMG22487	GPF-tagged wild-type of PsJN	this study
B. phytofirmans Δ*oxc*	LMG22487	Δ*oxc* mutant of PsJN	this study

B. phytofirmans (pin62)	LMG22487	dsRED-tagged Doxc mutant of PsJN	this study
B. phytofirmans Δ*oxc* (pBBR1MCS-2-gfpmut3-1)	LMG22487	GPF-tagged Doxc of PsJN	this study
B. plantarii	ATCC43733	*Oryza sativa*	
B. plantarii	ATCC43733TT	*Oryza sativa*	
B. plantarii	ATCC43733VV	*Oryza sativa*	
B. plantarii	LMG9035	*Oryza sativa*	
B. pyrrocinia	LMG21822	soil	
B. pyrrocinia	LMG14191	soil	
B. pyrrocinia	LMG21823	water	
B. sacchari	LMG19450	soil	
B. seminalis	LMG24067	clinical isolate	
B. sordidicola	LMG22029	*Phanerochaete sordida*	
B. stabilis	R-6270	CF patient	Gotschlich *et al*. 2001
B. stabilis	LMG14294	CF patient	
B. terricola	FN313521	rhizosphere	Gasser *et al*. 2009
B. terricola	LMG20594	soil	
B. thailandensis	LMG20219	soil	
B. tropica	LMG22274	roots	
B. tuberum	LMG21444	root nodules	
B. ubonensis	LMG20358	soil	
B. vietnamiensis	LMG18835	CF patient	
B. xenovorans	LMG21463	soil	
Escherichia coli	DH10B	Invitrogen Top10 cells	Invitrogen
Escherichia coli	MM294 (pRK2013)		Figurski and Helinski 1979, Nakagawa *et al*. 1996
Escherichia coli	DH5α	Invitrogen DH5α cells	Invitrogen
Escherichia coli	MT102 (pSB403)		Huber *et al*. 2003
Escherichia coli	CC118λpir		Herrero *et al*. 1990

Plasmid	**Specification**	**Characteristics**	**Reference**
pin62	DsRed donor plasmid	oripBBR mob+, Cmr, DSRed	Vergunst *et al*. 2010
pBBR1MCS-2-gfpmut3-1	GFP donor plasmid	pBAH7, KmR, GFP	Rothballer *et al*. 2005

Figure S1 (A) Structure of the gene cluster involved in oxalate degradation in *B. phytofirmans* PsJN (source: http://www.ncbi.nlm.nih.gov). Genes are located on chromosome 2. Bphyt_6739: putative oxalate transporter; Bphyt_6740: oxalate decarboxylase; Bphyt_6741: formyl CoA transferase. (B) Construction of a Δ*oxc* mutant in *B. phytofirmans* PsJN. The diagram illustrates the cloning steps involved in the mutagenesis of the *oxc* gene.

A



B

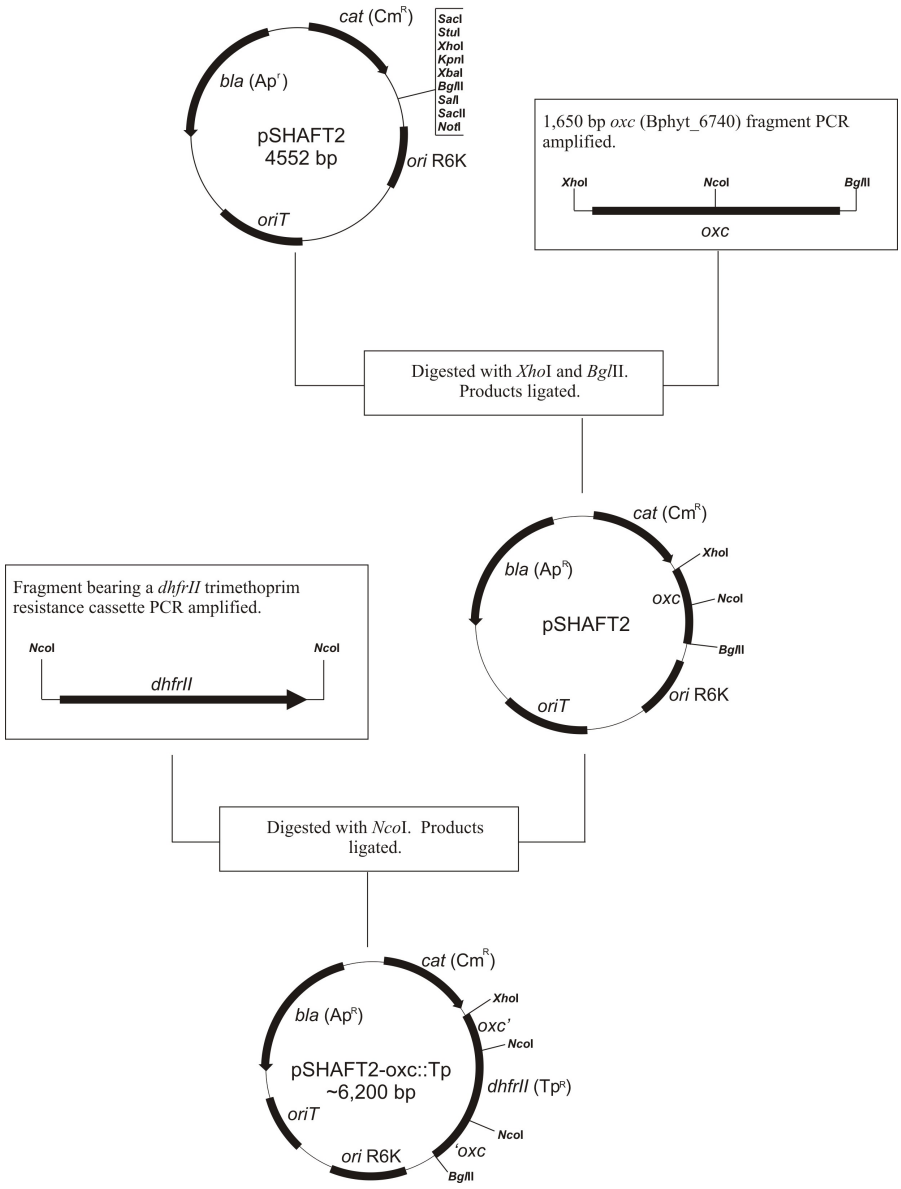
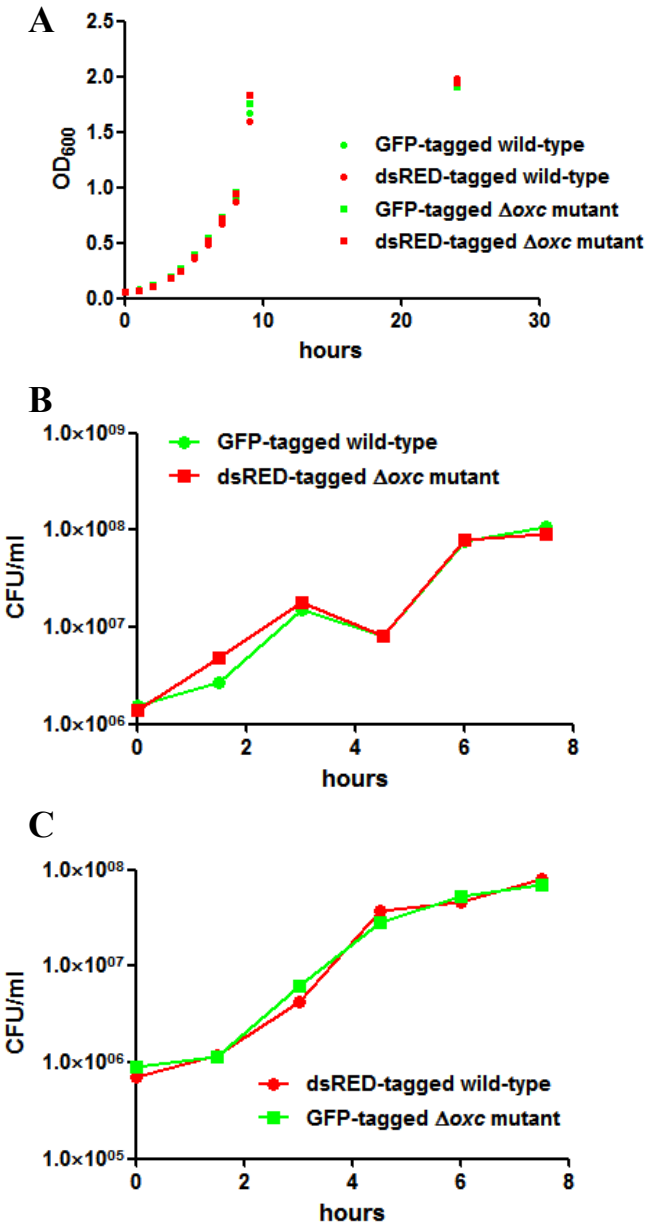

cat (Cm^R)
SacI
StuI
XhoI
KpnI
XbaI
BglII
SalI
SacII
NotI
bla (Ap^r)
pSHAFT2
4552 bp
ori R6K
oriT
1,650 bp oxc (Bphyt_6740) fragment PCR amplified.
XhoI
NcoI
BglII
oxc
Digested with XhoI and BglII. Products ligated.
Fragment bearing a dhfrII trimethoprim resistance cassette PCR amplified.
NcoI
NcoI
dhfrII
cat (Cm^R)
bla (Ap^R)
XhoI
oxc
NcoI
pSHAFT2
BglII
oriT
ori R6K
Digested with NcoI. Products ligated.
cat (Cm^R)
bla (Ap^R)
XhoI
oxc'
NcoI
pSHAFT2-oxc::Tp
~6,200 bp
dhfrII (Tp^R)
oriT
NcoI
ori R6K
'oxc
BglII

Figure S2 Growth curves and *in vitro* competition experiment of *B. phytofirmans* PsJN wild-type strain and Δ*oxc* mutant. (A) Optical densities of each strain grown as pure culture in LB. (B) Colony forming units (CFU) / ml of GFP-tagged wild-type and dsRED-tagged Δ*oxc* mutant when grown together. CFU/ml of dsRED-tagged wild-type and GFP-tagged Δ*oxc* mutant in mixed culture.



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The present thesis investigated three aspects of the ecology of *Burkholderia* species that had not yet been studied in detail: i) it brings new insights into the factors determining *Burkholderia* distribution in the soil and reveals their genus-wide acid tolerance, ii) it highlights the importance of interacting with fungi for the survival in the soil, and iii) it describes oxalotrophy as a new determinant of successful root colonization by plant-beneficial *Burkholderia*.

We were able to show that pH has a significant influence on the distribution of *Burkholderia* in the soil. Unlike most bacteria, *Burkholderia* species have a preference for moderately acidic soils and can hardly be detected in neutral or alkaline soils. Experiments testing the ability of a wide selection of *Burkholderia* strains to grow in media with different pH revealed the genus-wide acid tolerance of *Burkholderia* species. Moreover, the same experiments also showed that *Burkholderia* are not acidophilic but rather acidotolerant, as all tested *Burkholderia* strains were able to grow at pH 9 as well as at pH 4.5.

We used different methodologies to address the question of *Burkholderia* distribution in the soil. High-throughput sequencing is a well-described and frequently applied method for studying such questions, as it provides high depth coverage of the diversity and of the relative abundance of microbes in a given environment. However, most methods available at present produce only short sequencing reads, which greatly limits the phylogenetic depth at which sequences can be assigned, in most cases at the order, family, or genus level, very rarely at species level. This is the reason why such methods are not suitable to answer questions related to genus-based studies such as ours. For investigating the relative abundance of *Burkholderia*, we applied a quantitative PCR approach, whereas the analysis of intra-generic diversity was performed with Sanger sequencing, which yields long reads allowing species level assignment. Optimizing the qPCR protocol for the 16S rRNA primers previously described (Bergmark *et al*. 2012) allowed us to gain highly specific and efficiently amplified *Burkholderia* target products in DNA samples derived from single cultures and environmental samples. With this method, we were able to analyze soils across

continents covering a wide spectrum of ecosystems, which allowed us to gain a global view of the relative abundance of *Burkholderia* in soils. The results showed that *Burkholderia* relative abundance is negatively correlated with pH. This correlation was even more evident when analyzing a soil transect with a stable pH gradient, where a linear decrease in *Burkholderia* relative abundance was observed with increasing pH. Remarkably, *Burkholderia* were undetectable in neutral and alkaline soils at both sampling scales. This effect of pH on the relative abundance of *Burkholderia* was further demonstrated in microcosms where soil was acidified, which resulted in a significant increase in *Burkholderia* relative abundance. Analyzing the chemical properties of these microcosm soils allowed verifying that pH did not significantly influence the chemical composition of the soils, and in particular that acidification did not increase the availability of heavy metals. No correlation between the soil chemical composition and the relative abundance of *Burkholderia* was detected, suggesting that pH had a direct effect on *Burkholderia* relative abundance and does not act indirectly through changes in the soil chemical composition.

In contrast to the relative abundance of *Burkholderia*, diversity and community composition was not correlated to pH nor to any other environmental parameter. This could be explained by the large differences observed in the *Burkholderia* community composition between the different locations, of which only few (n=14) were investigated. However, it could also mean that pH indeed only affects relative abundance and not intra-generic diversity, since acid tolerance is a common feature of members of the *Burkholderia* genus and would likely not select for some species over others. We thus hypothesized that *Burkholderia* community composition in the soil could be significantly affected by biological interactions rather than by pH, as such interactions of *Burkholderia* with plants or fungi are well-described in the literature.

To test this hypothesis, we investigated in the second project the occurrence and the nature of interactions between fungi and *Burkholderia* and why such interactions might play an important role for *Burkholderia* in acidic soils. Strong co-occurrence of *Burkholderia* and fungi detected by co-occurrence analysis on a wide range of soils revealed that biological interactions are taking place in soils between the two types of

organisms and that it does not solely originate from a shared niche preference. Nazir *et al*. (2012) showed that the capacity to comigrate with *Lyophyllum* sp. in the soil is widespread among the genus *Burkholderia*. Here we extended this knowledge by including three more fungi, *A. alternata*, *F. solani* and *R. solani*, which were shown to co-occur with *Burkholderia*, and co-cultivating them with four different *Burkholderia* strains. The results confirmed earlier findings by Nazir *et al*. (2012) and suggested that these interactions have a significant (ecological) importance in the environment. Co-cultivations between *B. glathei* and the two fungi *A. alternata* and *F. solani* were further studied with proteomics, which enabled us to gain insight into putative metabolic benefits for *Burkholderia* and revealed possible ecological roles of such interactions. Our results indicate that the benefits *Burkholderia* gain from such interactions mainly reside in enhanced substrate availability, as proteins indicative of starvation for essential nutrients such as carbon, nitrogen and phosphate were significantly reduced or even absent in co-cultures, while they were detected in high amounts when *B. glathei* was cultivated alone. Interestingly, *B. glathei* repressed the formation of its mobility apparatus in the presence of both fungi, which suggests that it was relying on the fungal partner for transportation and exploration of new niches. However, despite these benefits, *B. glathei* apparently also experienced stressful conditions in the co-cultivations, since higher numbers of proteins involved in defense, tolerance and detoxification were observed when *B. glathei* was growing in the presence of either fungus than when it was growing alone. It appears likely that this capacity to tolerate and/or detoxify fungal defense compounds might be at least partly responsible for the successful colonization of fungal hyphae observed throughout the genus *Burkholderia*.

In addition to their interactions with fungi, *Burkholderia* species are well-known for their capacity to colonize the rhizosphere of plants. However, the factors enabling them to do so are largely unknown. The last project of this thesis addressed the putative role of oxalate degradation in the interactions between *Burkholderia* and plants, with a particular focus on root colonization. Oxalate can be very abundant in the soil and it is produced and/or released by a large number of plants. Studies have shown that species of *Burkholderia* are among those oxalotrophic bacteria that are highly enriched in the rhizosphere of oxalate-producing plants. However the link

between oxalate degradation and bacteria-plant interaction has never been investigated. Thus we tested a large number of *Burkholderia* strains for their ability to degrade oxalate. Results suggest that oxalotrophy is a feature that is maintained exclusively in the environmental, non-pathogenic group of *Burkholderia*. The involvement of oxalate degradation in the interaction of *Burkholderia* with plants was further investigated by comparing the colonization behaviour of a engineered mutant of *B. phytofirmans* impaired in oxalotrophy with that of the wild-type. The mutated strain showed significantly less efficient root colonization of maize and lupin, as well as reduced persistence in the plants compared to the wild-type. These results suggest that the plant-released oxalate might have an additional function to those described earlier in protection, regulation and detoxification: it could act as an attractant for plant beneficial bacteria from the surrounding. Beyond this putative chemotaxis effect, oxalate could also act selectively on those bacteria already present in the root vicinity, since this carboxylate can be toxic for the bacterial strains that are not able to metabolize it. Since fungi can also produce high quantities of oxalate, the ability to use, tolerate, and/or detoxify this compound could be of broader interest for *Burkholderia* strains and enable them to establish beneficial interactions with both plants and fungi.

Combining our results, we can conclude that the genus *Burkholderia* is an important member of the microbial communities living in acidic soils and that it has a large ecological potential. Their survival in acidic soils likely results from a combined ability to tolerate low pH, to detoxify and/or use metabolites exuded by other soil inhabitants such as fungi and plants and to establish beneficial interactions with them.

Outlook

The present thesis describes the wide ecology potential of the genus *Burkholderia* in the soil. However, we still do not know whether this potential is constantly active or whether it is exploited in particular conditions and if so, which conditions these are. T address this question, a study on the activity of *Burkholderia* in the soil would be necessary. Using metatranscriptomics or metaproteomics in the soil would provide relevant information on the activity of *Burkholderia* in such environment and with additional manipulations of soils, it could provide also valuable information on temporal processes in which *Burkholderia* are involved as well as on the triggering factors leading to the expression *Burkholderia*'s metabolic potential.

Additionally, our work showed that associations between *Burkholderia* and fungi are very common and highly important for survival of *Burkholderia* in the soil. However, the model system used in this thesis only represents a potential association and should be validated in future studies by investigating relevant model organisms that have been shown to associate also in the environment, such as the examples of *B. terrae* BS001 and *Lyophyllum* sp. Karst or *B. glathei* PML1(12) and *Scleroderma citrinum* (Nazir *et al.* 2010, Uroz *et al.* 2013). Furthermore, these studies should also investigate changes occurring in the fungal partners, which would provide a more complete view of *Burkholderia*-fungal interactions at the physiological level.

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Acknowledgments

It would not have been possible to write this doctoral thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

Above all, I would like to thank my wife **Dragana** for her personal support and great patience at all times. **My parents** and **sister** have given me their unequivocal support throughout, as always. I am forever indebted to you!

This thesis would not have been possible without the help, support, guidance and patience of my supervisor and mentor, **Dr. Laure Weisskopf**, not to mention her optimism during struggling times. I am extremely grateful to you for giving me the opportunity to work on this fascinating project in your group.

I would like to acknowledge **Prof. Dr. Leo Eberl** for welcoming me in his group, for his advices and support when it was most needed.

I also thank my thesis committee members; **Prof. Dr. Jakob Pernthaler** not only for reviewing my thesis but also for you contributions at the yearly progress reports; **Prof. Dr. Pilar Junier** for your willingness to review my thesis in short time and for the constructive discussions at various meetings.

I would like to acknowledge the academic and technical support of my collaborators. **Prof. Dr. Noah Fierer** for providing us the soil DNA samples and for his interest in our research. **Prof. Dr. Katharina Riedel** and **Dr. Daniela Zühlke** for performing the proteomics and your significant contribution to the proteome analysis and discussion. **Prof. Dr. Jim I. Prosser**, **Dr. Grame Nicol** and **Dr. Cecile Gubry-Rangin** for welcoming me in Aberdeen, providing the soil samples and for all the advices. **Dr. Beat Frey** for hosting me at the WSL Birmensdorf and help with the qPCR and soil analyses. **Dr. Natacha Bodenhausen** for help with R and diversity analyses. **Dr. Albert Barberán** for the co-occurrence network analysis and discussions.

I also thank **Dr. Aurélien Carlier** for his time and help on the genome sequencing, assembly and annotation as well as for the valuable discussions.

I would like to thank also all former and present members of the **Department of Microbiology** for all the great moments that we spent together in the lab and outside the lab. Thank you!

Last but not least I would like to thank also my friends. Chetan Viraktamath and Flurina Peyer, Christian Jenul and Katrin Schilcher, Gerardo Carcamo and Evelyn Avilés, Anoop and Anugraha Mathew thank you for all the laughs and chats, travels and dinners and all the moments that made my stay in Zürich unforgettable.

Appendix

List of transporters predicted from online tool TransAPP

Appendix Table 1. List of transporters predicted from the draft genome of *B. glathei* LMG14190 by TransAPP (http://www.membranetransport.org/). The transporters are listed by substrate/function predictions.

ORF Name	FID	Transporter Family	Subfamily	Substrate/Function
BGLT01830	GPTS	General PTS	EnzymeI	
BGLT04587	SSPTS	Sugar Specific PTS	EnzymeIIA	fructose
BGLT01831	SSPTS	Sugar Specific PTS	EnzymeIIABC	glucose/maltose/N-acetylglucosamine
BGLT01003	2-HCT	The 2-Hydroxycarboxylate Transporter (2-HCT) Family		sodium ion:citrate/malate symporter
BGLT00849	KDGT	The 2-Keto-3-Deoxygluconate Transporter (KDGT) Family		2-keto-3-deoxygluconate
BGLT03317	KDGT	The 2-Keto-3-Deoxygluconate Transporter (KDGT) Family		2-keto-3-deoxygluconate
BGLT00958	APC	The Amino Acid-Polyamine-Organocation (APC) Family		amino acid
BGLT02181	APC	The Amino Acid-Polyamine-Organocation (APC) Family		amino acid
BGLT04899	APC	The Amino Acid-Polyamine-Organocation (APC) Family		amino acid
BGLT06684	APC	The Amino Acid-Polyamine-Organocation (APC) Family		amino acid
BGLT04949	APC	The Amino Acid-Polyamine-Organocation (APC) Family		ethanolamine
BGLT05326	APC	The Amino Acid-Polyamine-Organocation (APC) Family		ethanolamine
BGLT00887	APC	The Amino Acid-Polyamine-Organocation (APC) Family		GABA
BGLT06350	APC	The Amino Acid-Polyamine-Organocation (APC) Family		GABA
BGLT03982	APC	The Amino Acid-Polyamine-Organocation (APC) Family		large neutral amino acid
BGLT04283	Amt	The Ammonia Transporter Channel (Amt) Family		ammonium
BGLT00275	Bestrophin	The Anion Channel-forming Bestrophin (Bestrophin) Family		Bestrophin anion channel
BGLT06169	Bestrophin	The Anion Channel-forming Bestrophin (Bestrophin) Family		Bestrophin anion channel
BGLT01664	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT01883	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT02032	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT02426	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT02772	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT04139	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT05141	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?
BGLT06741	ArAE	The Aromatic Acid Exporter (ArAE) Family		fusaric acid efflux?

BGLT01365	ACR3	The Arsenical Resistance-3 (ACR3) Family		arsenite
BGLT03575	ACR3	The Arsenical Resistance-3 (ACR3) Family		arsenite
BGLT00981	ArsB	The Arsenite-Antimonite (ArsB) Efflux Family		arsenite (ArsB)
BGLT00370	AAE	The Aspartate:Alanine Exchanger (AAE) Family		aspartate:alanine antiporter
BGLT00371	AAE	The Aspartate:Alanine Exchanger (AAE) Family		aspartate:alanine antiporter
BGLT00595	AAE	The Aspartate:Alanine Exchanger (AAE) Family		aspartate:alanine antiporter
BGLT02151	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	?
BGLT02338	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	?
BGLT02487	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	?
BGLT04429	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	?
BGLT00743	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	2-aminoethylphosphonate
BGLT01142	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	2-aminoethylphosphonate
BGLT02486	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	2-aminoethylphosphonate
BGLT02486	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	2-aminoethylphosphonate
BGLT05014	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	2-aminoethylphosphonate
BGLT00478	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT00813	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT00982	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT01123	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT01156	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT01195	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT01244	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT01632	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT02048	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT02061	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT03035	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT03250	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT03853	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT03917	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid

4341				(glutamine/glutamate/aspartate?)
BGLT04514	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT04724	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT04884	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT05088	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT05928	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT06015	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	amino acid (glutamine/glutamate/aspartate?)
BGLT00144	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino acid (lysine/arginine/ornithine/histidine/octopine)
BGLT00145	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino acid (lysine/arginine/ornithine/histidine/octopine)
BGLT02760	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino acid (lysine/arginine/ornithine/histidine/octopine)
BGLT02761	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino acid (lysine/arginine/ornithine/histidine/octopine)
BGLT00476	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00477	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00814	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00815	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00984	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00985	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT01628	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT01629	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT02046	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT02047	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT04515	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT04885	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT04886	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT05086	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)

BGLT05929	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT05930	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT06012	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT06013	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	amino aicd (glutamine/glutamate/aspartate?)
BGLT00335	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00336	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00482	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00483	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00689	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00690	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00705	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT00706	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01214	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01217	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01218	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01387	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01388	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT01393	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT02644	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	branched-chain amino acid
BGLT02646	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT02647	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03022	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03023	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03327	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03328	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03620	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03710	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT03711	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	branched-chain amino acid
BGLT05869	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT05922	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT05985	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	branched-chain amino acid
BGLT02989	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	carbohydrate
BGLT05409	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	carbohydrate
BGLT03962	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	choline
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	choline

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BGLT04000	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	choline
BGLT00299	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	cobalamin/Fe3+-siderophores
BGLT01944	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	cobalamin/Fe3+-siderophores
BGLT06608	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	cobalamin/Fe3+-siderophores
BGLT01537	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	cobalt
BGLT04848	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	cobalt
BGLT05639	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	cobalt
BGLT00367	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	D-methionine
BGLT00534	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	D-methionine
BGLT00535	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	D-methionine
BGLT02528	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	D-methionine
BGLT02529	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	D-methionine
BGLT05548	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	D-methionine
BGLT00334	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00396	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00481	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00687	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00688	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00703	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00704	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01091	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01212	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01213	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01220	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01221	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01385	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01386	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT01395	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT03021	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT03325	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT03326	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT03621	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT04669	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT05871	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT05920	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT05986	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin

BGLT05987	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT06233	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	daunorubicin
BGLT00364	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT00365	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT00366	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT00759	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT00760	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT00761	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT01504	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT01505	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT02921	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT02922	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT02923	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT03245	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT03246	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT03247	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT04146	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT04147	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT04148	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT05297	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT05298	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT05550	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT05551	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	dipeptide/oligopeptide
BGLT05552	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	dipeptide/oligopeptide
BGLT00135	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT00684	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT00762	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT00763	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT00891	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT01506	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT01579	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT02185	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT02187	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT02919	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT02920	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT03041	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine

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BGLT03733	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT03775	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT03776	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT03969	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT04003	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT04145	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT04486	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT04675	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT05295	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT05334	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT05336	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT05549	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT06049	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT06506	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	glycine betaine
BGLT02186	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	glycine betaine/L-proline
BGLT03774	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	glycine betaine/L-proline
BGLT04002	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	glycine betaine/L-proline
BGLT05335	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	glycine betaine/L-proline/carnitine/choline
BGLT05337	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	glycine betaine/L-proline/carnitine/choline
BGLT02740	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	heme
BGLT06672	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	heme
BGLT02741	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	heme export
BGLT02742	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	heme export
BGLT04705	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	heme export
BGLT00294	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	iron-hydroxamate
BGLT01942	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	iron-hydroxamate
BGLT06607	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	iron-hydroxamate
BGLT04156	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	iron(III)
BGLT04157	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	iron(III)
BGLT06616	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	iron(III)
BGLT06617	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	iron(III)
BGLT00323	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT00337	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT00941	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT01190	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine

BGLT01215	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT01216	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT01247	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT02184	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT02643	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT03550	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT03709	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT04743	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT05060	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT05989	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT06029	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	leucine/valine
BGLT02383	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipid A
BGLT01449	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipoprotein
BGLT04610	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipoprotein
BGLT05296	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipoprotein
BGLT05603	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipoprotein
BGLT06230	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	lipoprotein
BGLT01448	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	lipoprotein releasing
BGLT04611	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	lipoprotein releasing
BGLT06228	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	lipoprotein releasing
BGLT06229	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	lipoprotein releasing
BGLT00397	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	manganese/zinc ion
BGLT00536	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	methionine
BGLT02527	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	methionine
BGLT03741	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	methionine
BGLT04123	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	methionine
BGLT04304	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	mobybdenate
BGLT06313	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	mobybdenate
BGLT06311	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	molybdate
BGLT06312	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	molybdate
BGLT03083	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	multidrug
BGLT04680	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	multidrug
BGLT05484	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	multidrug
BGLT05485	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	multidrug
BGLT06234	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	multidrug
BGLT00892	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate

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BGLT03239	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT03252	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT04122	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT04320	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT04578	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT05499	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT05940	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT06021	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT06051	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	nitrate
BGLT00136	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT00683	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT00893	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT02975	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT02976	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT03220	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT03240	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT03241	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT03242	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT03251	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT04121	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT04318	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT04319	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT04485	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT04487	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT04577	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT05500	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT05941	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT06022	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT06024	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT06050	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT06052	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT06457	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	nitrate/sulfonate/taurine
BGLT06507	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	nitrate/sulfonate/taurine
BGLT01503	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	oligopeptide
BGLT05299	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	oligopeptide
BGLT00146	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate

BGLT0 0333	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 0475	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 0816	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 0983	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 1396	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 1627	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 2045	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 2762	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 3060	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 3061	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	phosphate
BGLT0 3062	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	phosphate
BGLT0 3712	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 3916	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 4428	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 4725	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 4888	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 5087	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 5931	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 6014	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphate
BGLT0 6129	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	phosphate
BGLT0 0298	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 1945	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 2645	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 3020	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 3622	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 5870	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 6280	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 6281	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 6282	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	phosphonate
BGLT0 6290	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	phosphonate
BGLT0 6291	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	phosphonate
BGLT0 6609	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonate
BGLT0 3226	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonates
BGLT0 3229	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonates
BGLT0 3253	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonates
BGLT0 6582	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	phosphonates
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine

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BGLT0 0388	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 0718	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 0746	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 0771	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 0986	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 1040	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 1143	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 1995	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 2462	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 2489	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 2562	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 2970	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 4158	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 4252	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 4687	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5020	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5067	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5289	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5290	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5404	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 5632	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 6222	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 6615	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	polyamine
BGLT0 1092	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	polysaccharide export
BGLT0 4668	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	polysaccharide export
BGLT0 1941	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	pyoverdin (siderophore) exporter PvdE
BGLT0 2663	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	pyoverdin (siderophore) exporter PvdE
BGLT0 2673	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	pyoverdin (siderophore) exporter PvdE
BGLT0 0036	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT0 1789	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT0 3255	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT0 3260	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT0 4606	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT0 4735	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose

BGLT05309	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT05567	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT06000	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT06573	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	rhamnose
BGLT03013	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	ribose
BGLT03461	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	ribose
BGLT04605	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	ribose
BGLT04607	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	ribose
BGLT05106	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	ribose
BGLT05753	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	ribose
BGLT00745	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT00768	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT00769	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT00770	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT00987	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT00988	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT01145	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02459	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02460	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02461	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT02488	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02561	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT02563	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02564	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02968	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT02969	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT02971	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT04251	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT04253	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT04254	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT05018	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT05019	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT05064	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT05065	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT05066	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT05630	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine

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BGLT05633	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	spermidine/putrescine
BGLT06219	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT06220	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	spermidine/putrescine
BGLT00154	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT00155	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT00156	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT00390	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT00391	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT00392	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT00716	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT00717	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT01036	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT01037	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT01038	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT01992	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT01993	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT01994	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT04104	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT04105	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT04107	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT04689	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT04690	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT04691	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT05406	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT05407	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT05408	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT05529	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	sugar
BGLT05530	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT05531	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT05532	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar
BGLT06510	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT06511	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar
BGLT06513	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar
BGLT00034	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT00035	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT01255	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)

BGLT01256	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT01790	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT01791	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT03014	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT03015	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT03257	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT03258	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT03259	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT03462	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT03463	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT04590	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT04591	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT04592	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT04604	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT04733	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT04734	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT05104	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05105	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT05307	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05308	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT05310	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05394	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT05395	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05396	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05565	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05566	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT05751	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT05752	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT06435	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT06570	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC	sugar (ribose?)
BGLT06571	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT06572	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (ribose?)
BGLT06436	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sugar (xylose?)
BGLT00099	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT00480	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT00744	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sulfate
BGLT0	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sulfate

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BGLT03219	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT05403	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT05476	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT05477	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sulfate
BGLT05478	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	sulfate
BGLT05921	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate
BGLT05479	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	sulfate/thiosulfate
BGLT04030	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	toluene tolerance
BGLT04671	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	toluene tolerance
BGLT04673	ABC	The ATP-binding Cassette (ABC) Superfamily	binding protein	toluene tolerance
BGLT04674	ABC	The ATP-binding Cassette (ABC) Superfamily	membrane	toluene tolerance
BGLT06489	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	toluene tolerance
BGLT03494	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	toxin secretion
BGLT03700	ABC	The ATP-binding Cassette (ABC) Superfamily	ABC+ membrane	toxin secretion
BGLT00055	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT00484	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT00691	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT00692	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT00707	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT01219	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT01390	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT01394	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT03323	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT03329	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT03619	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT05872	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT05923	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	urea
BGLT01257	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	xylose
BGLT04589	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	xylose
BGLT05393	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	xylose
BGLT06434	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	xylose
BGLT00395	ABC	The ATP-binding Cassette (ABC) Superfamily	binding	zinc
BGLT01170	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT02083	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT03789	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT04056	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export

BGLT05009	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT05557	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT06196	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT06522	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT06827	AI-2E	The Autoinducer-2 Exporter (AI-2E) Family (Formerly the PerM Family, TC #9.B.22)		Autoinducer-2 export
BGLT01341	AEC	The Auxin Efflux Carrier (AEC) Family		
BGLT04046	AEC	The Auxin Efflux Carrier (AEC) Family		
BGLT06331	AEC	The Auxin Efflux Carrier (AEC) Family		
BGLT00529	DNA-T	The Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family		
BGLT01451	DNA-T	The Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family		
BGLT04500	DNA-T	The Bacterial Competence-related DNA Transformation Transporter (DNA-T) Family		
BGLT02428	BenE	The Benzoate:H+ Symporter (BenE) Family		benzoate
BGLT04976	BenE	The Benzoate:H+ Symporter (BenE) Family		benzoate
BGLT02609	LIV-E	The Branched Chain Amino Acid Exporter (LIV-E) Family	AzlC	branched-chain amino acid efflux (AzlC)
BGLT03149	LIV-E	The Branched Chain Amino Acid Exporter (LIV-E) Family	AzlC	branched-chain amino acid efflux (AzlC)
BGLT04087	CaCA	The Ca2+:Cation Antiporter (CaCA) Family		proton:calcium ion antiporter
BGLT00501	CDF	The Cation Diffusion Facilitator (CDF) Family		cation efflux
BGLT00937	CDF	The Cation Diffusion Facilitator (CDF) Family		cation efflux
BGLT02523	CDF	The Cation Diffusion Facilitator (CDF) Family		cation efflux
BGLT03706	CDF	The Cation Diffusion Facilitator (CDF) Family		cation efflux
BGLT06438	CDF	The Cation Diffusion Facilitator (CDF) Family		cation efflux
BGLT00244	ClC	The Chloride Carrier/Channel (ClC) Family		chloride ion channel
BGLT04228	ClC	The Chloride Carrier/Channel (ClC) Family		chloride ion channel
BGLT04416	ClC	The Chloride Carrier/Channel (ClC) Family		chloride ion channel
BGLT04618	ClC	The Chloride Carrier/Channel (ClC) Family		chloride ion channel
BGLT06171	ClC	The Chloride Carrier/Channel (ClC) Family		chloride ion channel
BGLT01263	CHR	The Chromate Ion Transporter (CHR) Family		chromate ion
BGLT01264	CHR	The Chromate Ion Transporter (CHR) Family		chromate ion
BGLT01670	CHR	The Chromate Ion Transporter (CHR) Family		chromate ion
BGLT03895	CHR	The Chromate Ion Transporter (CHR) Family		chromate ion
BGLT05269	CHR	The Chromate Ion Transporter (CHR) Family		chromate ion
BGLT04570	CitMHS	The Citrate-Mg2+:H+ (CitM) Citrate-Ca2+:H+ (CitH) Symporter (CitMHS) Family		proton:citrate symporter
BGLT02367	MIT	The CorA Metal Ion Transporter (MIT) Family		magnesium/cobalt ion
BGLT02469	MIT	The CorA Metal Ion Transporter (MIT) Family		magnesium/cobalt ion
BGLT03644	MIT	The CorA Metal Ion Transporter (MIT) Family		magnesium/cobalt ion
BGLT05743	Oxa1	The Cytochrome Oxidase Biogenesis (Oxa1) Family		60 KD inner membrane protein OxaA homolog
BGLT0	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+)		proton/sodium

2153		Symporter (DAACS) Family		ion:glutamate/aspartate symporter
BGLT03480	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT04303	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT05025	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT05126	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT05315	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT06059	DAACS	The Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family		proton/sodium ion:glutamate/aspartate symporter
BGLT01207	DASS	The Divalent Anion:Na+ Symporter (DASS) Family		sodium ion:anion symporter
BGLT05056	DASS	The Divalent Anion:Na+ Symporter (DASS) Family		sodium ion:dicarboxylate/sulfate
BGLT04639	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	RarD	chloramphenicol (RarD homolog)
BGLT00675	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01109	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01139	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01248	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01400	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01545	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01578	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01668	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01979	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT02093	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT02416	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT02517	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT02727	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	DME	drug/metabolite?
BGLT03510	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT04390	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	DME	drug/metabolite?
BGLT04634	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT05556	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT05824	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT06047	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT06271	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT06730	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT06736	DMT	The Drug/Metabolite Transporter (DMT) Superfamily		drug/metabolite?
BGLT01046	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	SMR	multidrug efflux (SMR)

BGLT02336	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	SMR	multidrug efflux (SMR)
BGLT05511	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	SMR	multidrug efflux (SMR)
BGLT05645	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	SMR	multidrug efflux (SMR)
BGLT06278	DMT	The Drug/Metabolite Transporter (DMT) Superfamily	SMR	multidrug efflux (SMR)
BGLT03647	EVE1-C	The Envelope Virus E1 Channel (EVE1-C) Family		
BGLT03632	FNT	The Formate-Nitrite Transporter (FNT) Family		formate/nitrite
BGLT03501	GntP	The Gluconate:H+ Symporter (GntP) Family		gluconate
BGLT03779	GntP	The Gluconate:H+ Symporter (GntP) Family		gluconate
BGLT02150	ESS	The Glutamate:Na+ Symporter (ESS) Family		sodium ion:glutamate symporter
BGLT06832	ESS	The Glutamate:Na+ Symporter (ESS) Family		sodium ion:glutamate symporter
BGLT02719	Mot/Exb	The H+- or Na+-translocating Bacterial Flagellar Motor 1ExbBD Outer Membrane Transport Energizer (Mo		
BGLT02720	Mot/Exb	The H+- or Na+-translocating Bacterial Flagellar Motor 1ExbBD Outer Membrane Transport Energizer (Mo		
BGLT03486	Mot/Exb	The H+- or Na+-translocating Bacterial Flagellar Motor 1ExbBD Outer Membrane Transport Energizer (Mo		
BGLT03487	Mot/Exb	The H+- or Na+-translocating Bacterial Flagellar Motor 1ExbBD Outer Membrane Transport Energizer (Mo		
BGLT05231	Mot/Exb	The H+- or Na+-translocating Bacterial Flagellar Motor 1ExbBD Outer Membrane Transport Energizer (Mo		
BGLT01198	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01199	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01200	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01201	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01202	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01203	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01204	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01205	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT01206	F-ATPase	The H+- or Na+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily		protons
BGLT03417	HCC	The HlyC/CorC (HCC) Family		heavy metal ion
BGLT03359	HCC	The HlyC/CorC (HCC) Family		hemolysin C (HlyC) homolog
BGLT00264	TRIC	The Homotrimeric Cation Channel (TRIC) Family		
BGLT03335	PiT	The Inorganic Phosphate Transporter (PiT) Family		phosphate
BGLT05655	PiT	The Inorganic Phosphate Transporter (PiT) Family		phosphate
BGLT02711	ILT	The Iron/Lead Transporter (ILT) Superfamily		iron ion
BGLT06333	ILT	The Iron/Lead Transporter (ILT) Superfamily		iron ion
BGLT04460	KUP	The K+ Uptake Permease (KUP) Family		potassium ion uptake
BGLT04386	LysE	The L-Lysine Exporter (LysE) Family		amino acid efflux
BGLT04729	LctP	The Lactate Permease (LctP) Family		L-lactate
BGLT06496	LctP	The Lactate Permease (LctP) Family		L-lactate
BGLT06735	LctP	The Lactate Permease (LctP) Family		L-lactate
BGLT0	MscL	The Large Conductance Mechanosensitive Ion Channel		large-conductance

5525		(MscL) Family		mechanosensitive ion channel
BGLT05874	FeT	The Low Affinity Fe2+ Transporter (FeT) Family		
BGLT00485	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT01225	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT01966	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT03719	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT03903	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT04083	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT04719	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT06089	MFS	The Major Facilitator Superfamily (MFS)		4-hydroxyphenylacetate
BGLT05690	MFS	The Major Facilitator Superfamily (MFS)		Acetyl-CoA:CoA antiporter
BGLT03045	MFS	The Major Facilitator Superfamily (MFS)		cyanate
BGLT00109	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT00883	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT01096	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT01270	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT01368	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT01766	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT02958	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT03782	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT03836	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT03949	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT04103	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT04175	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT04409	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT04561	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT04747	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT05320	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT05828	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT05993	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06009	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06048	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06074	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06090	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06180	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06250	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT0	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate

6253				
BGLT06415	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06564	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06643	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT06698	MFS	The Major Facilitator Superfamily (MFS)		D-galactonate
BGLT02817	MFS	The Major Facilitator Superfamily (MFS)		glycerol-3-phosphate
BGLT03073	MFS	The Major Facilitator Superfamily (MFS)		glycerol-3-phosphate
BGLT04600	MFS	The Major Facilitator Superfamily (MFS)		glycerol-3-phosphate
BGLT00030	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT00033	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT00552	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT00655	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT00818	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01163	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01499	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01639	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01641	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01697	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT01699	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT02984	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT02992	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT02993	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT03718	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT03986	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT04311	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT04974	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT04975	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT05262	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT05981	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT06020	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT06397	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT06398	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT06687	MFS	The Major Facilitator Superfamily (MFS)		metabolite (benzoate?)
BGLT00160	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT00242	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT00379	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT00380	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux

BGLT0 0498	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0601	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0656	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0659	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0700	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0750	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0846	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0874	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0940	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0944	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 0998	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1330	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1353	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1402	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1476	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1532	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1688	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 1762	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2091	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2126	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2165	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2172	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2313	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2493	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2551	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2553	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2786	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2848	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 2876	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3029	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3525	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3547	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3582	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3882	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 3941	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0 4305	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT0	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux

4394				
BGLT04536	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT04955	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT04990	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT05160	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT05248	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT05285	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT05913	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT05968	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06006	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06062	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06081	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06082	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06132	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06191	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06336	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06346	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06448	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06475	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06493	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06520	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06587	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06599	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06626	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06640	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT06721	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux
BGLT00114	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT00663	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT01022	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT01159	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT01807	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT02688	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT03434	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)

BGLT03713	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT04186	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT04810	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05022	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05265	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05280	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05488	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05727	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT05850	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT06177	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT06325	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT06531	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux (EmrB/QacA subfamily)
BGLT00729	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux?
BGLT03213	MFS	The Major Facilitator Superfamily (MFS)		multidrug efflux?
BGLT04266	MFS	The Major Facilitator Superfamily (MFS)		nitrate
BGLT05373	MFS	The Major Facilitator Superfamily (MFS)		nitrate
BGLT05374	MFS	The Major Facilitator Superfamily (MFS)		nitrate
BGLT00257	MFS	The Major Facilitator Superfamily (MFS)		nitrate/nitrite
BGLT01308	MFS	The Major Facilitator Superfamily (MFS)		nitrate/nitrite
BGLT02329	MFS	The Major Facilitator Superfamily (MFS)		nitrate/nitrite
BGLT03933	MFS	The Major Facilitator Superfamily (MFS)		nitrate/nitrite
BGLT00833	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT00859	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT01285	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT02114	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT05282	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT05292	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT05816	MFS	The Major Facilitator Superfamily (MFS)		oxalate:formate antiporter
BGLT00041	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT00970	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT0	MFS	The Major Facilitator Superfamily (MFS)		sugar

2407				
BGLT02628	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT02842	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT03214	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT03996	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT05894	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT05937	MFS	The Major Facilitator Superfamily (MFS)		sugar
BGLT06691	MFS	The Major Facilitator Superfamily (MFS)		tetracycline efflux?
BGLT03871	MSS	The Malonate:Na+ Symporter (MSS) Family		sodium ion:malonate symporter
BGLT03872	MSS	The Malonate:Na+ Symporter (MSS) Family		sodium ion:malonate symporter
BGLT00878	Nramp	The Metal Ion (Mn2+-iron) Transporter (Nramp) Family		manganese/iron ion
BGLT01355	Nramp	The Metal Ion (Mn2+-iron) Transporter (Nramp) Family		manganese/iron ion
BGLT05233	Nramp	The Metal Ion (Mn2+-iron) Transporter (Nramp) Family		manganese/iron ion
BGLT05634	Nramp	The Metal Ion (Mn2+-iron) Transporter (Nramp) Family		manganese/iron ion
BGLT06262	Nramp	The Metal Ion (Mn2+-iron) Transporter (Nramp) Family		manganese/iron ion
BGLT04230	MgtE	The Mg2+ Transporter-E (MgtE) Family		magnesium ion
BGLT05782	CPA1	The Monovalent Cation:Proton Antiporter-1 (CPA1) Family		potassium/sodium ion:proton antiporter
BGLT04398	CPA1	The Monovalent Cation:Proton Antiporter-1 (CPA1) Family		sodium ion:proton antiporter
BGLT00011	CPA2	The Monovalent Cation:Proton Antiporter-2 (CPA2) Family		potassium/sodium ion:proton antiporter
BGLT00493	CPA2	The Monovalent Cation:Proton Antiporter-2 (CPA2) Family		potassium/sodium ion:proton antiporter
BGLT05782	CPA2	The Monovalent Cation:Proton Antiporter-2 (CPA2) Family		potassium/sodium ion:proton antiporter
BGLT00741	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MATE	multidrug efflux
BGLT02094	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MATE	multidrug efflux
BGLT02836	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MATE	multidrug efflux
BGLT03156	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MATE	multidrug efflux
BGLT04497	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MATE	multidrug efflux
BGLT00431	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	PST	polysaccharide export
BGLT00456	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	PST	polysaccharide export
BGLT00463	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	PST	polysaccharide export
BGLT03279	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	PST	polysaccharide export
BGLT04207	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	PST	polysaccharide export
BGLT05904	MOP	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	MVF	virulence factor MviN
BGLT06831	NhaA	The NhaA Na+:H+ Antiporter (NhaA) Family		sodium ion:proton antiporter
BGLT06503	NiCoT	The Ni2+-Co2+ Transporter (NiCoT) Family		nickel ion
BGLT00256	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/ thiamine/allantoin
BGLT04169	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/ thiamine/allantoin
BGLT04630	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/ thiamine/allantoin

BGLT04740	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/thiamine/allantoin
BGLT04959	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/thiamine/allantoin
BGLT04963	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/thiamine/allantoin
BGLT05543	NCS1	The Nucleobase:Cation Symporter-1 (NCS1) Family		cytosine/purines/uracil/thiamine/allantoin
BGLT00190	NCS2	The Nucleobase:Cation Symporter-2 (NCS2) Family		xanthine/uracil
BGLT01079	NCS2	The Nucleobase:Cation Symporter-2 (NCS2) Family		xanthine/uracil
BGLT05304	NCS2	The Nucleobase:Cation Symporter-2 (NCS2) Family		xanthine/uracil
BGLT01897	OOP	The OmpA-OmpF Porin (OOP) Family		
BGLT05232	OOP	The OmpA-OmpF Porin (OOP) Family		
BGLT06184	OOP	The OmpA-OmpF Porin (OOP) Family		
BGLT03495	OMF	The Outer Membrane Factor (OMF) Family		
BGLT02546	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT02547	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT03414	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT03415	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT05772	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT05774	MTB	The Outer Membrane Protein Secreting Main Terminal Branch (MTB)		
BGLT02399	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT02400	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT02401	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT03749	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT03750	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT03751	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT06537	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT06538	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT06539	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		potassium ion
BGLT01293	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		zinc/cadmium/cobalt ion
BGLT02820	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		zinc/cadmium/cobalt ion
BGLT03567	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		zinc/cadmium/cobalt ion
BGLT05236	P-ATPase	The P-type ATPase (P-ATPase) Superfamily		zinc/cadmium/cobalt ion
BGLT02484	PNaS	The Phosphate:Na+ Symporter (PNaS) Family		sodium ion:phosphate symporter
BGLT00172	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT01915	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT02178	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT02321	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT02475	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT0	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux

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BGLT02871	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT03660	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT04386	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT04613	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT05713	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT05748	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT05927	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT06535	RhtB	The Resistance to Homoserine/Threonine (RhtB) Family		amino acid efflux
BGLT03571	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HME	cobalt/zinc/cadmium ion efflux (HME subfamily)
BGLT00206	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily		multidrug efflux
BGLT01023	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily		multidrug efflux
BGLT01663	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily		multidrug efflux
BGLT00794	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT01312	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT01921	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT02018	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT02536	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT02585	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT03737	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT03738	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT04375	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT04421	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT04623	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT04875	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT05366	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT06140	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT06158	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT06213	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1

				subfamily)
BGLT06214	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT06805	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT06806	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE1	multidrug/solvent efflux (HAE1 subfamily)
BGLT04031	RND	The Resistance-Nodulation-Cell Division (RND) Superfamily	HAE2	multidrug/solvent efflux (MmpL homolog/HAE2 subfamily)
BGLT03585	AtoE	The Short Chain Fatty Acid Uptake (AtoE) Family		short-chain fatty acid
BGLT06126	AtoE	The Short Chain Fatty Acid Uptake (AtoE) Family		short-chain fatty acid
BGLT00159	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT00347	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT00963	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT00976	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT02006	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT02524	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT02819	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT04138	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT05574	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT06799	MscS	The Small Conductance Mechanosensitive Ion Channel (MscS) Family		small-conductance mechanosensitive ion channel
BGLT01345	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT01349	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT01419	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT01925	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT02070	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT02402	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT02425	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT02730	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT02941	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT03057	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT03752	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT05709	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT0	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline

6103				symporter
BGLT06499	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT06529	SSS	The Solute:Sodium Symporter (SSS) Family		sodium ion:proline symporter
BGLT00052	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT00610	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT01012	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT01673	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT02940	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT04995	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT05005	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT05063	SulP	The Sulfate Permease (SulP) Family		sulfate
BGLT02620	TerC	The Tellurium Ion Resistance (TerC) Family		tellurium ion efflux
BGLT05338	TerC	The Tellurium Ion Resistance (TerC) Family		tellurium ion efflux
BGLT00555	TDT	The Telurite-resistance/Dicarboxylate Transporter (TDT) Family		tellurite
BGLT01616	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT01617	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT01678	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT01679	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT03803	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT03804	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT05958	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT05959	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT06296	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT06297	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT06298	TRAP-T	The Tripartite ATP-independent Periplasmic Transporter (TRAP-T) Family		C4-dicarboxylate
BGLT04651	Tat	The Twin Arginine Targeting (Tat) Family		protein export
BGLT04652	Tat	The Twin Arginine Targeting (Tat) Family		protein export
BGLT04653	Tat	The Twin Arginine Targeting (Tat) Family		protein export
BGLT00715	IISP	The Type II (General) Secretory Pathway (IISP) Family		preprotein translocase SecY subunit
BGLT01058	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT01227	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT01228	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT02932	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT02933	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT02934	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT02935	IIISP	The Type III (Virulence-related) Secretory Pathway (IIISP) Family		
BGLT03106	IVSP	The Type IV (Conjugal DNA-Protein Transfer or VirB) Secretory Pathway (IVSP) Family		

BGLT04868	IVSP	The Type IV (Conjugal DNA-Protein Transfer or VirB) Secretory Pathway (IVSP) Family		
BGLT05883	IVSP	The Type IV (Conjugal DNA-Protein Transfer or VirB) Secretory Pathway (IVSP) Family		
BGLT00872	UT	The Urea Transporter (UT) Family		urea
BGLT00012	VIT	The Vacuolar Iron Transporter (VIT) Family		vacuolar iron uptake transporter homolog
BGLT00013	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT00609	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT01660	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT02442	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT03785	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT04614	VIC	The Voltage-gated Ion Channel (VIC) Superfamily		potassium ion channel
BGLT00178	YggT	The YggT or Fanciful K+ Uptake-B (FkuB; YggT) Family		?

Conference proceedings

Nejc Stopnisek, Natacha Bodenhausen, Beat Frey, Noah Fierer, Leo Eberl, Laure Weisskopf: *Genus-wide acid tolerance accounts for the biogeographical distribution of soil* Burkholderia *populations* (poster). BAGECO12, Ljubljana, Slovenia; 06/2013.

Nejc Stopnisek, Natacha Bodenhausen, Beat Frey, Noah Fierer, Leo Eberl, Laure Weisskopf: *Biogeography of soil* Burkholderia *populations* (poster). ISME14, Copenhagen, Denmark; 08/2012.

Nejc Stopnisek, Natacha Bodenhausen, Beat Frey, Noah Fierer, Leo Eberl, Laure Weisskopf: *Biogeography of soil* Burkholderia *populations* (oral presentation). SME 2013, Murten, Switzerland; 02/2012.

Nejc Stopnisek, Noah Fierer, Leo Eberl, Laure Weisskopf: *Influence of acidity on the abundance and diversity of soil* Burkholderia *populations* (poster). Ecology of Soil Microorganisms, Prague, Czech Republic; 05/2011.

Teaching experience

2011 - 2013 Planning of a project and supervision of student groups during the yearly block course Bio284

2011 - 2013 Teaching in the yearly undergraduate course Bio132

2011 Work supervision of Master Student on the project Oxalotrophy in the genus *Burkholderia*